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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A23L 1/00 — A2

(11) International Publication Number: WO 00/47062

(43) International Publication Date: 17 August 2000 (17.08.00)

(21) International Application Number: PCT/US00/02687

(22) International Filing Date: 1 February 2000 (01.02.00)

(30) Priority Data:

09/243,197	1 February 1999 (01.02.99)	US
09/364,248	29 July 1999 (29.07.99)	US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: ENTERIC FORMULATIONS OF PROANTHOCYANIDIN POLYMER DIETARY SUPPLEMENTS AND METHODS FOR PREPARING SAME

(57) Abstract

The present invention provides for a method for preparing a proanthocyanidin enriched composition useful as a dietary supplement. The proanthocyanidin polymer composition can be synthesized by the method comprising the steps of precipitating *Croton ssp* latex by adjusting the pH of the latex; removing precipitated residue from the precipitated latex to produce a filtrate; concentrating the filtrate to obtain a retentate; and drying the filtrate, the filtrate being essentially free of anti–foaming agents. A further optional additional step includes removing additional taspine from the retentate by contacting said retentate with chromatographic media. A proanthocyanidin composition product made by this process is also described. Dietary supplements containing a proanthocyanidin polymer enriched composition as well as dietary supplements containing a proanthocyanidin polymer enriched composition and an additional herbal agent, e.g., ginger, cinnamon, and peppermint oil are also described.

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ENTERIC FORMULATIONS OF PROANTHOCYANIDIN POLYMER DIETARY SUPPLEMENTS AND METHODS FOR PREPARING SAME

This application claims priority benefits of Application No. 09/243,197, filed February 1, 1999 and Application No. 09/364,248, filed July 29, 1999, the entire disclosures of which are incorporated herein by reference.

1. FIELD OF INVENTION

The present invention relates to dietary supplements and methods for preparing the same, and in particular enterically coated formulations for colonic delivery containing enriched proanthocyanidin polymer concentrates derived from Croton latex. In addition, the present invention relates to dietary supplements, and in particular proanthocyanidin enriched extracts and additional herbal agents for mammals suffering from gastrointestinal disorders; methods for using and processes for obtaining the same.

2. BACKGROUND OF THE INVENTION

Citation or identification of any reference in Section 2 or any other section of this application shall not be construed as an admission that such reference is available as prior art.

2.1 GASTROINTESTINAL DISORDERS

2.1.1 IRRITABLE BOWEL SYNDROME

Irritable bowel syndrome (IBS) is a condition that effects an estimated 22 million Americans each year, and accounts for 50% of referral to gastroenterologists. The condition is present with varying symptoms, including abdominal cramps, abdominal pain, diarrhea, constipation and excessive flatulence. There can also be increased intestinal gas, nausea and loss of appetite. While some have thought the condition to be caused by irritability of the intestinal tract, it has not been well elucidated, and treatment primarily focuses on symptomatic relief. Current treatment options include high fiber diets, bulking agents, muscle relaxants, psychotherapy, and even antidepressants. Thompson, W.G., *Amer. J. Gastroenterol* 81(2), 95 (1986). Shaw, G., et al., *Digestion* 50:36 (1991).

2.1.2 SECRETORY DIARRHEA

Secretory diarrheas, also called watery diarrheas, are a major source of illness and mortality in developing countries, particularly in infants and young children and also affect a significant proportion of visitors from developed to developing countries and

can also affect any person visiting a foreign country (called "traveler's diarrhea"). Secretory diarrhea is characterized by the loss of both fluid and electrolytes through the intestinal tract, leading to serious and often life-threatening dehydration. Secretory diarrhea is caused by a variety of bacterial, viral and protozoal pathogens and also results from other non-infectious etiologies such as ulcerative colitis, inflammatory bowel syndrome, and cancers and neoplasias of the gastrointestinal tract. In fact, it is believed that all types of diarrheal disease may have a secretory component.

V. cholerae, the enterotoxigenic strains of *E. coli*, and a variety of other enteric bacteria elicit secretory diarrhea via similar mechanisms. These pathogens produce a toxin which binds a specific receptor on the apical membrane of the intestinal epithelium. Binding of the receptor triggers an adenylate cyclase- or guanylate cyclase-mediated signal transduction leading to an increase in cAMP or cGMP. This regulatory cascade, apparently acting through phosphorylation of specific apical membrane proteins, stimulates chloride efflux into the gut from the intestinal epithelial crypt cells and inhibits normal resorption of sodium and chloride ions by the intestinal epithelial villus cells. The increased chloride and sodium ion concentration osmotically draws water into the intestinal lumen, resulting in both dehydration and loss of electrolytes. Agents which reduce chloride ion secretion will, therefore, prevent the fluid movement into the intestine and resulting net fluid elimination. Thus, such agents are particularly useful for treating and preventing the dangerous dehydration and electrolyte loss associated with secretory diarrhea.

The dietary supplement compositions of the present invention are particularly useful for administration to mammals suffering from traveler's diarrhea and non-specific diarrhea to normalize gastrointestinal function. Traveler's diarrhea, which is a type of secretory diarrhea, is defined as diarrhea experienced by citizens of industrialized nations who travel to "third world" countries. An example of traveler's diarrhea is diarrheal disease experienced by United States citizens that travel to Mexico for the first time and have diarrhea within the 3-5 days of arrival (Castelli & Carose, *Chemotherapy* 4(supp. 1), 20-32 (1995)). Bacteria are estimated to be responsible for 85% of traveler's diarrhea with enterotoxigenic *Escherichia coli* (ETEC), *Shigella* spp., and *Campylobacter jejuni* being the principal etiologic agents. Protozoa and viruses also cause traveler's diarrhea but at lower frequencies than bacteria (DuPont, *Chemotherapy* 4(supp. 1), 33-39 (1995)). In Mexico, in the summer months (May to November), the predominant etiologic agent associated with traveler's diarrhea is ETEC, whereas in the winter months, the principal organism is Campylobacter jejuni (DuPont, "Traveler's diarrhea", M. Blaser et al., eds., pp. 299-311, Raven Press, New York (1995). Approximately 40% of first time United States travelers to Mexico experience traveler's diarrhea.

In contrast to traveler's diarrhea, non-specific diarrhea (NSD), which also appears to have a secretory component, is an acute endemic diarrheal disease experienced by indigenous populations. The attack rate of non-specific diarrhea in Mexican residents is 7% (H.L. DuPont, personal communication). Unlike traveler's diarrhea, however, non-specific diarrhea generally does not respond to antibiotic therapy and the etiology is not known.

Secretory diarrheas are also associated with viral infections, such as, diarrheas which accompany Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS), and rotavirus infection, in particular. Almost all AIDS patients suffer from diarrhea at some point during the course of the disease, and 30% of AIDS patients suffer from chronic diarrhea. The diarrhea that accompanies AIDS has been termed "HIV-Associated Chronic Diarrhea." This diarrheal component of HIV disease is thought to be caused, at least in some patients, by a secondary infection of protozoal pathogens, particularly Cryptosporidium spp. Additionally, rotavirus infection is a substantial cause of diarrhea particularly in infants and young children in developing countries.

Secretory diarrhea is also a significant problem in non- human animals, particularly in farm animals, such as bovine animals, swine, sheep (ovine animals), poultry (such as chickens), and equine animals, and other domesticated animals such as canine animals and feline animals. Diarrheal disease is particularly common in young and recently weaned farm animals. Diarrheal disease in farm animals, particularly food animals such as cattle, sheep and swine, is often caused by bacterial pathogens such as enterotoxigenic, enterohemorrhagic and other *E. coli*, *Salmonella* spp., *Clostridium perfringens*, *Bacteriodes fragilis*, *Campylobacter* spp., and *Yersinia enterocolitica*. Additionally, protozoal pathogens, particularly *Cryptosporidium parvum*, and viral agents, particularly rotaviruses and coronaviruses, are significant causes of diarrhea in farm animals. Other viral agents which have been implicated in diarrhea of farm animals include togavirus, parvovirus, calicivirus, adenoviruses, bredaviruses, and astroviruses. See generally Holland, *Clin. Microbiology Rev.* 3, 345 (1990) ; see also Gutzwiller and Blum, *AJVR* 57, 560 (1996); Strombeck, *Veterinary Quarterly* 17(Supp. 1), S12 (1995); Vermunt, *Austral. Veterinary J.* 71,33 (1994); Driesen et al., *Austral. Veterinary J*, 70:259, (1993) Mouricout, *Eur. J. Epidemiol.* 2, 588 (1991); Ooms and Degryse, *Veterinary Res.* Comm. 10, 355 (1986).

2.2 ANTI-OXIDANT ACTIVITY

The antioxidant properties of phenolic compounds, such as vitamin E (a non-flavonoid monophenolic compound) and especially polyphenols, such as proanthocyanidins, are well-documented. These compounds are free radical scavengers in biological systems. The oxygen molecule (O_2) is involved in the respiratory process under normal conditions. Under certain conditions, however, it can be transformed into superoxide anion (O_2), hydroxyl radical (OH), singlet oxygen (O_2 dg), and/or hydrogen peroxide (H_2O_2). The superoxide anion, the hydroxyl radical, and specifically, singlet oxygen (free radicals), are responsible for anemia and aging (leathery-looking skin), and stress (prostate problems, etc.). Proanthocyanidins are also known to protect cells from lipid peroxidation, resulting in the protection of target organs' membranes. An example is the protection of low density lipoproteins (LDL; so-called bad cholesterol) from oxidation. The oxidation of LDL is a contributing factor to atherosclerosis and cardiovascular disease. A variety of proanthocyanidins have been effective in preventing the growth of breast cancer cells. Proanthocyanidins are very potent free radical scavengers and metal chelators. They reduce free radicals, a by-product of metabolism and block their propagation. The complexity of the proanthocyanidins results from their biological diversity.

2.3 PLANT EXTRACTS CONTAINING TANNINS OR PROANTHOCYANIDINS AND USE AGAINST DIARRHEA

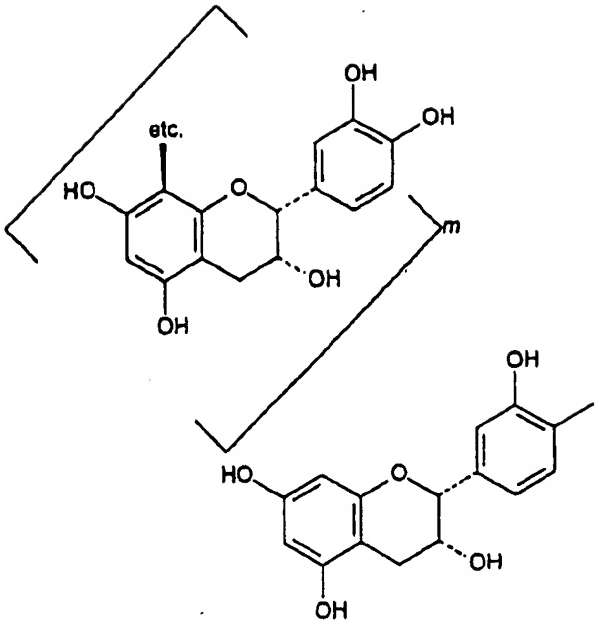
Tannins are found in a wide variety of plants and are classified as either hydrolyzable or condensed. Proanthocyanidins are a group of condensed tannins and are described further below. Many members of *Croton spp* have been used in traditional medicine (See *e.g.*, Van de Berg, MA., *Advances in Economic Botany Ethnobotany in Neotropics*, GT Prance eds., New York Botanical Garden, Bronx, NY., pp. 40-149 (1984); Heinrich, M., et al, *J. Ethnopharmacol* 36(1), 63-80 (1992). Many plants used in traditional medicine as treatment or prophylaxis for diarrhea have been found to contain tannins and proanthocyanidins in particular (*see, e.g.*, Yoshida et al., *Phytochemistry* 32, 1033 (1993); Yoshida et al., *Chem. Pharm. Bull.*, 40, 1997 (1992); Tamaka et al., *Chem. Pharm. Bull.* 40, 2092 (1992). Crude extracts from medicinal plants, for example, *Pycanthus angolenis* and *Baphia nitida*, have been shown to have antidiarrheal qualities in animal tests (Onwukaeme and Anuforo, *Discovery and Innovation*, 5, 317 (1993); Onwukaeme and Lot, *Phytotherapy Res.*, 5,254 (1991)). Crude extracts which contain tannins, in particular extracts from carob pods and sweet chestnut wood, have been proposed as treatments or prophylactics for diarrhea (U.S. Patent No. 5,043,160; European Patent No.481,396).

Crude plant extracts containing proanthocyanidins have also been proposed as treatments or prophylactics for diarrhea. For example, crude fruit skin extracts, which contain anthocyanidins as well as other compounds, have been suggested for use against diarrhea (U.S. Patent No. 4,857,327). The bark from the *Q. petrea* tree, traditionally used against diarrhea, has been shown to contain oligomeric proanthocyanidins (Konig and Scholz, , *J. Nat. Prod.*, 57, 1411 (1994); Pallenbach, *Planta Med.*, 59, 264 (1993)). A fraction of *Sclerocarya birrea* bark extract, which also contains procyanidins, reduced the intestinal contractions associated with experimentally-induced diarrhea (Galvez et al., *Phyt. Res.*, 7, 25 (1993); Galvez et al., *Phyt. Res.*, 5, 276 (1991)). However, none of these studies demonstrate that the proanthocyanidins are specifically responsible for the antidiarrheal activity of the extracts.

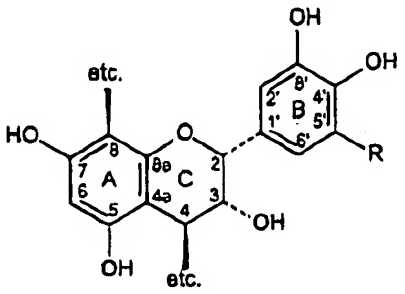
Proanthocyanidins have different physiological effects, depending on their structure and source. Other proanthocyanidins are actually contraindicated for treatment or prevention of diarrhea. Oligomeric proanthocyanidins isolated from black bean were shown to increase chloride secretion and reduce sodium resorption in isolated intestinal tissue [Silverstein, 1989, "Procyanidin from Black Bean (Phaseolus Vulgaris): Effects on Transport of Sodium, Chloride, Glucose, and Alanine in the Rat Ileum," Washington State University (Dissertation)]. The increased ion concentration in the intestine would thus promote fluid accumulation in the intestinal lumen and aggravate the fluid and electrolyte loss and dehydration associated with secretory diarrhea. In fact, the reference specifically teaches away from the use of proanthocyanidins as a treatment for diarrhea and suggests that the proanthocyanidins might cause secretory diarrhea.

2.4 PROANTHOCYANIDINS

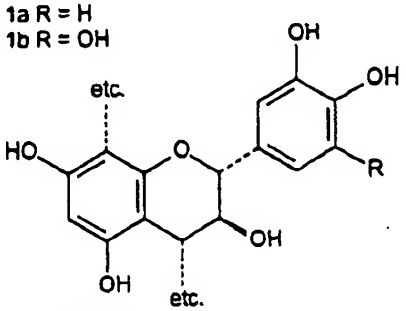
Proanthocyanidin and proanthocyanidin polymers are phenolic substances found in a wide variety of plants, particularly those with a woody habit of growth (*e.g.*, *Croton* spp. and *Calophyllum* spp.). The general chemical structure of a polymeric proanthocyanidin consists of linear chains of 5, 7, 3', 4' tetrahydroxy or 5, 7, 3', 4', 5' pentahydroxy flavonoid 3-ol units linked together through common C(4)-(6) and/or C(4)-4' C(8) bonds, as shown below.



Biosynthetic studies have indicated that proanthocyanidin polymers consist of monomer units of the type shown below. *See* Fletcher et al., *J.C.S. Perkin*, 1: 1628 (1977).



1a R = H
1b R = OH



2a R = H
2b R = OH

The monomer unit (generally termed "leucoanthocyanidin") of the polymer chain may be based on either of two stereo-chemistries of the C-ring, at a 2 and/or 4 position designated cis (called epicatechins) or trans (called catechin). Leucoanthocyandins may also be in the form of gallocatechins, galloepicatechins, flavanols, flavan-3, 4-diols, leucocyanidins and anthrocyanidins. Therefore, the polymer chains are based on different structural units, which create a wide variation of polymeric proanthocyanidins and a large number of possible isomers (Hemingway et al., *J.C.S. Perkin*, 1, 1217 (1982)). ^{13}C NMR has been useful to identify the structures of polymeric proanthocyanidins and recent work has elucidated the chemistry of di-, tri- and tetra-meric proanthocyanidins. Larger polymers of the flavonoid 3-ol units are predominant in most plants, and are found with average molecular weights above 2,000 daltons, containing 6 or more units (Newman, et al., *Mag. Res. Chem.*, 25, 118 (1987)).

2.5 ETHNOBOTANICAL USES OF EXTRACTS AND COMPOUNDS FROM *CROTON* AND *CALOPHYLLUM* SPECIES

A number of different *Croton* tree species, including *Croton sakutaris*, *Croton gossypifolius*, *Croton palanostima*, *Croton lechleri*, *Croton erythrochilus* and *Croton draconoides*, found in South America, produce a red viscous latex sap called Sangre de Drago or "Dragon's Blood". Sangre de Drago is most often utilized by mixed descent and native people of the Peruvian Amazon for flu and diarrhea. It is taken internally for tonsillitis, throat infections, tuberculosis, peptic ulcers, intestinal disorders, rheumatism and to enhance fertility and is used by both adults and children. It is also used extensively to stop bleeding, for herpes virus lesions, and for wound healing. The sap is placed directly on open wounds as an anti-infective and to accelerate the healing process. It is also utilized as a vaginal wash in cases of excessive bleeding.

It has been shown that Sangre de Drago from *Croton draconoides* and from *Croton lechleri* contains an alkaloid identified as taspine, which exhibits anti-inflammatory activity (Persinos, et al., *J. Pharm. Sci.*, 68, 124 (1979); U.S. Patent No. 3,694,557). Taspine has also been shown to inhibit RNA-directed DNA polymerase activity in the avian myeloblastosis virus, Rauscher leukemia virus and Simian sarcoma virus (Sethi, *Canadian J. Pharm. Sci.*, 12, 7 (1977)).

A variety of phenolic and diterpene compounds isolated from Sangre de Drago were tested for their antitumor, antibacterial and wound healing properties (Chen, et al., *Planta Med.*, 60, 541). The proanthocyanidins in the sap were found to have little antitumor or antibacterial activity and slight wound healing activity.

U.S. Patent No. 5,211,944 first described the use of the composition as an antiviral agent (See also Ubillas, et al., *Phytomedicine*, 1, 77 (1994)). The proanthocyanidin polymer composition was shown to have antiviral activity against a variety of viruses including respiratory syncytial, influenza, parainfluenza and herpes viruses.

Calophyllum inophylum is a tree ranging from India to East Africa to Polynesia. Seed oil is used in folk medicine as an antiparasitic in treatment of scabies, ringworm and dermatosis as well as other uses such as analgesia. In Indo-China, the powdered resin is used for ulcers and wound healing. In Indonesia, the bark is applied externally to treat swollen glands and internally as a diuretic. The sap is used as an emollient for chest pain as well as for tumors and swelling. Leaf extracts are used as a wash for inflamed eyes. The Cambodians use leaf extracts in inhalations for treatment of vertigo and migraine. The Samoans use the sap as an arrow poison.

U.S. Patent No. 5,211,944 also discloses the use of this composition as an antiviral agent.

It has been reported that the proanthocyanidin polymer compositions are acid labile and subject to inactivation by the acidic environment of the stomach. International Publication WO 98/1611 discloses an enterically coated pharmaceutical composition of a proanthocyanidin polymer composition isolated from either *Croton* spp. or *Calophyllum* spp. which is protected from the acidity of gastric fluid so that the proanthocyanidin polymer composition can be administered orally for treatment of secretory diarrhea.

2.6 METHODS OF PREPARING PROANTHOCYANIDIN EXTRACTS

In light of these proanthocyanidin polymers reported beneficial uses, there have been numerous methods reported for extracting or isolating proanthocyanidins from plant material. U.S. Patent No. 5,211,944 described the extraction of an aqueous soluble proanthocyanidin composition from *Croton* ssp. with water, a C_1-C_3 alcohol or acetone. Various organic solvents have been reported useful in the preparing extracts enriched in the proanthocyanidin material. Some of the organic solvents used include acetone, methanol, butanol, isopropanol, ethyl acetate, methylene chloride and dichloroethane. Some of these organic solvents, especially butanol and isopropanol, also provide the additional benefit of reducing the foaming which occurs with extracts containing saponins and sugars, as extracts from *Croton* are known to contain. However, these organic solvents present problems both in their use and disposal.

There are some problems is using these organic solvents. Some of these solvents are unsafe. For example, chlorinated hydrocarbons are known carcinogens.

Methanol, butanol and isopropanol are unfit for human consumption. While the need to remove such compounds is known, it is not practical to totally eliminate methanol and ethyl acetate in the presence of water without forming an azeotropic mixture.

U.S. Patent No. 5,762,936 describes the preparation of extracts from Leguminosae, *e.g.*, *Lens esculenta*, using volatile solvents of four or fewer carbons,(*e.g.*, methanol, ethanol and acetone). Alternatively, a hot water extract was precipitated with sodium chloride.

U.S. Patent No. 5,912,363 describes the preparation of extracts from plant material by contacting a permeate containing proanthocyanidins with absorbant material and then eluting the proanthocyanidins retained on the resin with a polar solvent. U.S. Patent No. 5,646,178, describes the preparation of extracts from Vaccinium by extracting with a polar solvent, alcohols with fewer than eight carbons. A metal acetate or sulfate was added to the extract to precipitate out the active fraction in a metal complex.

U.S. Patent No. 5,650,432 describes the preparation of extracts from *Croton* which includes the steps of adjusting the pH to about 10, mixing with methanol to form an active agent enriched precipitate, acidifying the resulting precipitate with 12 M hydrochloric acid and applying the acidified solution to a lipophilic column.

Konowalchuk, J, et al (*Applied & Environmental Microbiology* 35(6), 1219 (1978)) described the pH adjustment of fruit extracts to pH 7.0, but no mention of a precipitate is made.

Foo, et al, (Phytochemistry 19, 1749 (1980) described methanol as a poor solvent for proanthocyanidin polymers and described the use of LH-20 adsorption chromatography and Me_2CO-H_2O mixtures as powerful solvents.

Czochanska, et al, *J.C.S.Perkins Trans.* 1, 2278 (1980) described the use of Sephadex G-50 in acetone-water and Sephadex LH-20 to isolate proanthocyanidins from plant material.

Industrial scale-ups often render extraction procedures suitable for smaller scale, impractical. For example, while saturated salt solutions have been reported useful as precipitating agents, the quantities of salt necessary would be unwielding. Furthermore, such procedure would result in high residual salt levels in the extract, requiring their removal. Using multiple types of chromatographic media to isolate and extract the desired proanthocyanidins can require large amounts of expensive media. The use of organic solvents to elute materials off of such chromatographic media also increases the production costs. Furthermore, use of these solvents requires not only the environmental concerns attendant with the actual use of the material, but also disposal licenses and costs.

Extracting proanthocyanidins from *Croton* creates additional problems. Taspine is an alkaloid also found in *Croton*. While taspine has been reported useful as anti-inflammatory compositions, it also has other side effects which makes it undesirable. Thus any dietary supplement or pharmaceutical composition derived from *Croton* needs to remove or reduce the amount of taspine also extracted from the plant material. Methods to extract taspine from *Croton* have been reported. See U.S. Patent No. 3,694,557. Taspine is insoluble in water and alkaline solutions. Taspine extracts have been prepared as acid salts, *e.g.*, taspine hydrochloride, wherein the taspine is converted to an acid salt by the bubbling of the extract with hydrochloric acid. USP 3,694,557. However, the proanthocyanidin polymers of interest are acid labile and thus the exposure of the proanthocyanidins to an acidic environment is not desired. Alternatively, chlorinated hydrocarbons are used to extract taspine from an aqueous solution that has been made alkaline with the addition of caustic. Chlorinated hydrocarbons have the same problems as discussed earlier.

Thus there is a need for a method for preparing an enriched proanthocyanidin composition from Croton which reduces the taspine levels of Croton while maintaining the compositions usefulness as a dietary supplement. There is a need for a process for preparing an enriched proanthocyanidin composition from Croton which is essentially free from the use of environmentally undesirable organic solvents which cannot be removed easily from the final proanthocyanidin composition, especially chlorinated solvents or butanol. There is a need for a method which, rather than extracting the active proanthocyanidins from the undesired material, removes the undesired material from a concentrate at a reduced production cost. Applicants' present invention satisfies these needs.

2.7 HERBAL COMPOSITIONS/COMPOUNDS AND USE AGAINST GASTROINTESTINAL DISORDERS

2.7.1 GINGER

Ginger (*Zingiber officale*), is a plant native to Asia that is in the *Zingiberaceae* family, and is cultivated in the United States, India, China, West Indies, and tropical regions. PDR for Herbal Medicines, Medical Economics Co., Inc., Montvale, N.J., pp. 1229-1231 (1998). The characteristic odor and flavor of ginger root comes from a volatile oil composed of shogaols and gingerols. O'Hara, MA., et al., *Arch Fam Med*, 7:523 (1998). Ginger root has been reported as an antispasmodic to increase the tone and peristalsis of the intestines. PDR for Herbal Medicines, (1998), to treat hyperemesis gravidarum (Fischer-Rasmaussen, et al., *Eur J Obstet Gynecol Reprod. Biol.*, 38(1):19-24 (1991). As with peppermint oil, there have been inconsistent reports of ginger extracts

efficacy as anti-emesis agents, O'Hara, M., et al., *Arch Fam Med*, 7(6):523 (1998) to reduce nausea. Sharma, S.S., et al., *J Ethnopharmacol*, 57(2):93-6 (1997); Bone, M.E., et al., *Anaesthesia* 45:669 (1999); Phillips, S., et al., *Anaesthesia* 48:715 (1993); Visalyaputra, S., et al., *Anaesthesia* 53:486 (1998); Arfeen Z., et al., *Anaesth Intes Care* 23:449 (1995); and to treat motion sickness (Mowrey., D.B., Lancet __:655 (1982); Holtmann, S., et al., *Acta Pharmacology* 42:111 (1942). Yamahara also indicated that shogaols enhanced gastrointestinal motility. Yamahara, J., *Chem Pharm Bull* (Tokyo), 38(2):430 (1990). However, the inventors are unaware of any prior art which describes the use of this herbal agent in combination with a proanthocyanidin extract as a dietary supplement.

2.7.2 CINNAMON

Cinnamon comes from the tree bark of *Cinnamonomum aromaticum* Nees (= *C. Cassia*) and *C. verum* J. Presl (*C. zeylandicum*). *Cinnamonum verum* is indigenous the Sri Lanka and Southwest India (Mabberley, D.J., 1997). The Plant Book, Cambridge University Press, pp. 158-159). *Cinnamonomum aromaticum* is indigenous and cultivated in southern China, Vietnam and Burma. PDR for Herbal Medicines, Medical Economics Co., Inc., Montvale, N.J., pp. 749-753 (1998)). The chief compounds found in the volatile oil include cinnamaldehyde, weiterhin, cinnamylacetate, cinnamyl alcohol, o-methoxy cinnamaldehyde, cinnamic acid, courmarin, tannins, oligomere proanthocyanidins, and mucilages. Cinnamon has been used to treat loss of appetite, dyspeptic complaint and used generally in the symptomatic treatment of gastrointestinal disorders. [Schultz, V., et al., *Rational Phytotherapy*, Springer-Verlog, Berlin, pp. 24-26 (1997)].

Antiulcerogenic compounds have been isolated from *Cinnamomum cassia* = *C. aromaticum* (Shirage, Y., et al., *Tetrahedron* 44(15):4703 (1988); Tanaka, S., et al., *Planta Med* 55(3):245 (1989); and Akira, T., et al., *Planta Med*, 59(6):433 (1993). *O*-methoxycinnamaldehyde isolated from cinnamon has been reported to have antibiotic activity. Morozumi, S., *Applied and Environmental Microbiology* 36(4):577 91978). Cinnamon extracts were shown to inhibit growth of Helicobacter pylori and inhibit urease activity of Helicobacter pylori (Neeman, I., Tabak, M.; Armon, R., EP-689842-A1, filed 29 June 1994, Publication Date 03 January 1996). However, the inventors are unaware of any prior art which describes the use of this herbal agent in combination with a proanthocyanidin extract as a dietary supplement.

2.7.3 PEPPERMINT OIL

Medicinal peppermint (*Mentha piperita*; family *Lamiaceae*) is a hybrid perennial plant that no longer grows in the wild. Peppermint oil can be obtained by steam distillation of aerial parts of the flower *Mentha piperita (Lamiaceae)*. The active principle of peppermint oil is menthol, a cyclic monoterpene. Crude peppermint drugs, those with powdered or finely cut plant parts, are thought to contain at least 1.2% volatile oil to be effective. These volatile oils also contain 6-12% tannins along with flavonoids, triterpenes, and bitter principles.

There have been numerous reported biological uses of peppermint oil. Taylor has reported peppermint oil effective in relaxing isolated gastrointestinal smooth muscle of both animal and human colon. Taylor, E.A., et al., *Gut*, 24:A992 (1983) and Taylor, B.A., et al., *Gut*, 25:A1168 (1984). A fixed peppermint oil/caraway oil combination was reported to treat non-ulcer dyspepsia. May, B., et al., *Arzneimittelforschung*, 46(12):1149 1996). There are conflicting reports as to peppermint oil's efficacy in treating IBS in humans. See Pittler, M.H., et al., *Amer. J. Gastroenterol* 93(7):1131 (1998); Nash, P., et al., *J Clin Pract* 40(7):292 (1986); Thompson, W.G., (1986); Dew, M.J., et al., *Br J. Blin Pract*, 38(11/12):394, 398, (1984); and Rees, W.D., et al., *Br Med J.*, 2(6194):835, (1979). However, the inventors are unaware of any prior art which describes the use of this herbal agent in combination with a proanthocyanidin extract as a dietary supplement.

3. SUMMARY OF THE INVENTION

The present invention encompasses dietary supplement compositions useful for administration to mammals, including humans, suffering from gastrointestinal disorders comprising a proanthocyanidin polymer enriched concentrate (or extract), said concentrate comprising at least 35 % proanthocyanidins, and less than 2 % taspine, wherein said concentrate (or extract) is preferably derived or obtained from *Croton ssp* latex by a method which comprises:

(a) precipitating *Croton ssp* latex by adjusting the pH of the latex;

(b) removing precipitated residue from the precipitated latex to produce a filtrate;

(c) concentrating the filtrate to obtain a retentate; and

(d) drying the retentate, the retentate being essentially free of anti-foaming agents.

In one embodiment, the taspine level is further lowered by including another step of contacting said retentate with chromatographic media which removes taspine from the retentate.

In an alternative embodiment, the proanthocyanidin enriched extract is obtained from a *Croton* spp. by a method which comprises the steps of:

(a) obtaining latex from said *Croton* spp;

(b) precipitating with a solvent selected from the group consisting of water and a first short chain alcohol, said latex obtained from said *Croton* spp;

(c) extracting with a second short chain alcohol, a liquid retentate of the precipitating step;

(d) extracting the aqueous retentate from said second short chain alcohol extraction step on a solid phase extraction resin; and

(e) pooling the fractions eluted from the solid phase extraction resin.

In another alternative embodiment, the process for obtaining proanthocyanidin enriched extract comprises the steps:

separating latex obtained from a *Croton* tree into a solid phase and a liquid phase;

precipitating said liquid phase from said separating step with a solvent selected from the group consisting of water and isopropyl alcohol;

extracting with n-butanol, a liquid retentate from said liquid phase precipitating step;

concentrating said liquid aqueous retentate from said n-butanol extracting step by ultrafiltration;

extracting an aqueous retentate from said concentrating step on a solid phase extraction resin; and

pooling the fractions eluted from said solid phase extraction resin.

The present invention also encompasses dietary supplement compositions useful for administration to mammals, including humans suffering from gastrointestinal disorders comprising a proanthocyanidin enriched extract for colonic delivery; and at least one other herbal compound selected from the group consisting of ginger, cinnamon, and peppermint oil. The proanthocyanidin polymer composition is preferably prepared from a *Croton* spp., more preferably from *Croton lechleri*.

The dietary supplements embodying the present invention are formulated in part to protect the proanthocyanidin enriched polymeric composition from degradation by the acidic conditions of the stomach and from interactions with proteins, such as pepsin, present in the stomach. In certain embodiments, the dietary supplements of the invention

are formulated in part to protect the proanthocyanidin enriched extract and the additional herbal agents that need protection (*e.g.*, peppermint oil) from degradation by the acidic conditions of the stomach and from interactions with proteins, such as pepsin, present in the stomach; and to concurrently enable at least one additional herbal agent (ginger or cinnamon) to be released simultaneously to the stomach. In a preferred embodiment, the proanthocyanidin enriched extract is enteric coated, while another at least one additional herbal agent, *e.g.*, ginger or cinnamon, is formulated for delivery to the stomach.

In another embodiment, the method of preparing an enriched proanthocyanidin polymer concentrate useful as a dietary supplement comprises the steps:

(a) precipitating *Croton ssp* latex by adjusting the pH of said latex;

(b) removing precipitated residue from the precipitated latex to produce a filtrate obtained from the precipitating step;

(c) concentrating the filtrate from the precipitating step to obtain a retentate; and

(d) drying the retentate filtrate, the retentate filtrate being essentially free of anti-foaming agents. The dried retentate filtrate is characterized by a proanthocyanidin weight of at least 35% and a taspine level of less than 2 %.

In another embodiment, the invention additionally lowers taspine levels to at most 1% by weight by including the additional step of removing taspine from the retentate by contacting said retentate with a chromatographic media which is capable of removing taspine from the retentate to obtain a retentate filtrate.

In another embodiment, the present invention also encompasses methods for supplementing the diet of warm blooded animals or mammals, including humans suffering from gastrointestinal disorders, for example, diarrhea, both secretory and non-secretory in nature, upset stomach, particularly irritable bowel syndrome, comprising administering, to a non-human animal or human suffering from gastrointestinal disorders, a dietary supplement comprising a proanthocyanidin polymer concentrate derived from a *Croton* spp., or a *Calophyllum* spp., latex sufficient to normalize gastrointestinal function, formulated to protect the proanthocyanidin polymer composition from the stomach environment, *e.g.*, from the action of stomach acid and interaction with proteins, such as pepsin in the stomach, and a pharmaceutically acceptable carrier.

In another embodiment, the present invention also encompasses methods for supplementing the antioxidant levels of warm blooded animals or mammals, including humans, comprising administering, to a non-human animal or human suffering from gastrointestinal disorders, a dietary supplement comprising a proanthocyanidin polymer concentrate derived from a *Croton* spp., or a *Calophyllum* spp. latex, formulated to protect

the proanthocyanidin polymer composition from the stomach environment, *e.g.*, from the action of stomach acid and interaction with proteins, such as pepsin in the stomach, and a pharmaceutically acceptable carrier.

In addition, the present invention encompasses methods for supplementing the diet of animals suffering from irritable bowel syndrome in animals, including humans, comprising administering, to a non-human animal or human suffering from irritable bowel syndrome, (a) a dietary supplement comprising of the enriched proanthocyanidin polymer concentrate derived from a *Croton* spp., or a *Calophyllum* spp. latex, and a pharmaceutically acceptable carrier; and (b) a pharmaceutical composition either comprising an amount effective to inhibit stomach acid secretion of a compound that is effective to inhibit stomach acid secretion or comprising an amount effective to neutralize stomach acid of a compound that is effective to neutralize stomach acid, and a pharmaceutically acceptable carrier.

The present invention also provides a dietary supplement for administration to warm blooded animals, including humans, suffering from diarrhea or at risk of developing diarrhea, an amount of an enriched proanthocyanidin polymer concentrate derived from a *Croton* spp., or a *Calophyllum* spp. latex, formulated to protect the proanthocyanidin polymer composition from the stomach environment, and a pharmaceutically acceptable carrier.

In still another embodiment, the present invention also encompasses methods for supplementing the diet, or the treatment of warm blooded animals, including mammals, non-human primates and humans suffering from gastrointestinal disorders, for example, excessive flatulence, diarrhea, upset stomach, particularly irritable bowel syndrome, comprising administering, to a mammal, non-human primate or human suffering from gastrointestinal disorders, a dietary supplement comprising a therapeutically effective amount of a proanthocyanidin enriched extract isolated from a *Croton* spp., or a pharmaceutically acceptable derivative thereof, formulated to protect the proanthocyanidin enriched extract from the stomach environment, *e.g.*, from the action of stomach acid and interaction with proteins, such as pepsin in the stomach, and a pharmaceutically acceptable carrier in combination with at least one additional substance selected from ginger, peppermint oil and cinnamon. In addition, the present invention encompasses methods for supplementing the diet of and/or treating irritable bowel syndrome in animals, including humans, comprising administering, to a mammal, non-human primate or human suffering from irritable bowel syndrome, (a) a dietary supplement comprising a therapeutically effective amount of a proanthocyanidin enriched extract isolated from a *Croton* spp., or a pharmaceutically acceptable derivative thereof, and a pharmaceutically acceptable carrier;

and (b) a pharmaceutical composition either comprising an amount effective to inhibit stomach acid secretion of a compound that is effective to inhibit stomach acid secretion or comprising an amount effective to neutralize stomach acid of a compound that is effective to neutralize stomach acid, and a pharmaceutically acceptable carrier.

In a preferred embodiment, the method or process of obtaining a proanthocyanidin enriched extract useful as a dietary supplement comprises the steps: (1) obtaining the latex from the *Croton* tree; (2) separating the latex of the *Croton* tree into a liquid and solid phase; (3) precipitating the liquid phase from the separating sep with a solvent (*e.g.*, water or a first short chain alcohol, preferably n-butanol; (5) concentrating the aqueous phase of the second alcohol extracting step by ultrafiltration; (6) extracting the aqueous retentate from the ultrafiltration step on a solid phase extraction resin, preferably ion-exchange or absorption; and (7) pooling the fractions collected from the size exclusion column tha contain material with a UV detectable absorbance.

4. DETAILED DESCRIPTION OF THE INVENTION

4.1 PREPARATION OF THE ENRICHED PROANTHOCYANIDIN POLYMER COMPOSITION

In one embodiment, the dietary supplement, useful to normalize gastrointestinal functions of mammals suffering from various gastrointestinal disorders, is comprised, in part, of a concentrate enriched of proanthocyanidin polymers comprised of monomeric units of leucoanthocyanidins. Leucoanthocyanidins are generally monomeric flavonoids which include catechins, epicatechins, gallocatechins, galloepicatechins, flavanols, flavonols, and flavan-3,4-diols, leucocyanidins and anthocyanidins. The proanthocyanidin polymer composition useful for treating secretory diarrhea is comprised of polymers of 2 to 30 flavonoid units, preferably 2 to 15 flavonoid units, more preferably 2 to 11 flavonoid units and most preferably an average of 7 to 8 flavonoid units with a number average molecular weight of approximately 2500 daltons. The proanthocyanidin polymer composition is preferably soluble in an aqueous solution.

With regard to the novel proanthocyanidin polymer enriched concentrate which form part of the basis of this invention, such polymers are present in a concentrate derived from the latex of *Croton* tree or *Calophyllum* species by using the following method:

(a) precipitating the latex by adjusting the latex pH;

(b) removing precipitated residue from the precipitated latex to produce a filtrate obtained from the precipitating step;

(c) concentrating the filtrate from the precipitating step to obtain a retentate; and

(d) drying the retentate filtrate, the retentate filtrate including saponins and saccharides and essentially free of anti-foaming agents. The novel concentrate can be characterized by , ultraviolet radiation absorbance, at least 35% by weight proanthocyanidins, a moisture content of less than 15 %, preferably between 4 and 12% and has a taspine level of less than 2 % (20,000 parts per million).

Another embodiment of the present invention includes the additional step of removing additional taspine from the retentate by contacting the retentate with a chromatographic media which is capable of removing taspine from the retentate to obtain a retentate filtrate. The additional step lowers the taspine levels to less than 1% by weight (10,000 parts per million ["ppm"]).

The enriched proanthocyanidin polymer concentrate composition used in the present invention is preferably obtained from a *Croton spp.* or *Calophyllum* spp. The composition can be obtained using the entire tree or plant, the bark or preferably from the plant latex.

According to another preferred embodiment of the present invention, the novel proanthocyanidin polymer extract can be prepared as follows:

4.1.1 THE LATEX

The proanthocyanidin enriched fraction is preferably obtained from the latex of a *Croton* tree. While *Croton lecheri* is preferred, other Crotons which can be used include *Croton sakutaris*, *Croton gossypifolius*, *Croton palanostima*, *Croton erythrochilus* and *Croton draconoides*. Latex can be obtained from a *Croton* tree by scoring the tree and collecting the latex accumulating within the scores over a period of time, for example, 24 hours. The latex can be used directly, or can be separated into a liquid supernatant phase and a solid sediment phase. One way to effect this separation is to allow the latex to sit undisturbed for a period of time, for example between 2-28 days. The latex can be refrigerated to facilitate the settling, for example between 2°-8° C. Another method is to centrifuge the latex, pelleting the solids. By whatever method, the resulting liquid phase is carefully removed or siphoned off so as not to disturb the solid sediment and limit the amount of solid sediment removed.

4.1.2 PRECIPITATION PHASE

During this phase, the latex is precipitated without using saturated salt solutions and organic solvents, especially C_1-C_8 alcohols, ketones, ethers, esters and chlorinated hydrocarbons. To precipitate the latex and remove undesired materials, including taspine, some sugars and other glycosides, the pH of the latex, the liquid phase if separated from the settled out solid phase, is adjusted to neutral, for example to a range between 6.0 to 8.0 and preferably between 6.5 - 7.5 and most preferably between 7.0 and 7.2. Precipitating the latex by adjusting the pH, provides a liquid filtrate that is essentially free from additional (as used herein, the term "essentially free from additional . . ." is intended to mean that the composition contains less than 1% of the recited material): alkali metal halide salt solutions, *e.g.*, saturated sodium chloride and potassium chloride solutions; metal salts, *e.g.*, metal sulfates (lead sulfate) and acetates (acetates of zinc, magnesium, nickel, barium, calcium, cobalt, lead and sodium); chlorinated solvents, *e.g.*, dichloromethane, chloroform, trichloroethane, etc.; and C_1-C_8 alcohols, *e.g.*, methanol, ethanol, butanol, isopropanol, pentanol, and hexanol; ketones, *e.g.*, aliphatic ketones, *e.g.*, ethylethyl ketone, methylpropyl ketone, butylmethyl ketone; unsaturated ketones, *e.g.*, methylvinyl ketone, methylheptenone; alicyclic ketones, *e.g.*, cyclohexanone, cyclopentanone; aromatic ketones, *e.g.*, acetophenone, benzophenone; and heterocyclic ketones, *e.g.*, acetothienone); and ethers (simple aliphatic ethers, *e.g.*, ethyl ether, propyl ether, butyl ether; aliphatic mixed ethers, methylpropyl ether, methylethyl ether; aliphatic unsaturated ethers, *e.g.*, vinylether, allylether; aromatic ethers, *e.g.*, anisole, phenylbenzyl ether; and cyclic ethers, *e.g.*, ethylene oxide, and tetrahydrofurans). It is noted that none of the materials mentioned in the immediately preceding sentence is added using this preferred method for precipitation and hence the resulting composition is essentially free from these additional materials. Since the pH of raw Croton latex is below neutral, less than 7, usually between 4 and 5, the pH is adjusted upwards by using the appropriate base. Bases useful in the adjustment of the pH include, but are not limited to, sodium hydroxide, potassium hydroxide, lithium hydroxide, sodium bicarbonate and potassium bicarbonate. Care is taken to maintain the temperature of the latex constant when the base is added to the *Croton* latex.

4.1.3 SEPARATING PHASE

The resulting precipitated latex can then separated into a filtrate and residue by filtering to remove undesired solid impurities therefrom and to afford a crude filtrate containing the proanthocyanidins. Suitable filtering methods include passing the crude

extract through diatomaceous earth, *e.g.*, "CELATOM" TM brand of diatomaceous earth sold by Great Western Chemical located in Richmond, (CA); "CELITE" TM brand of diatomaceous earth sold by Fisher Scientific, Inc. located in Los Angeles, (CA); silica gel; or a fritted funnel. Centrifugation of solutions or diluted solutions of the crude extract can also be employed to remove undesired solid impurities therefrom.

4.1.4 CONCENTRATING PHASE

The resulting crude filtrate can then be ultrafiltered to concentrate the crude filtrate by removing water, solubilized impurities and other compounds smaller than the membrane cut-off size therefrom, contained in the permeate to afford a filtrate containing the proanthocyanidin polymers. Suitable concentrating methods include passing the crude filtrate through a 500 to 3000 dalton cellulose-based membrane, preferably a 1 kilodalton ultrafilter, or, a polypropylene or teflon membrane of comparable pore and cut-off size. Preferably the membrane used is a ultrafilter sold under the tradename PROSTAK™ by Millipore of Bedford, MA.

4.1.5 SEPARATORY PHASE

The resulting retentate can be contacted with chromatographic media to remove additional taspine from the retentate. This additional step is useful in methods for preparing proanthocyanidin extracts suitable for use in dietary supplements taken over an extended period of time, for example greater than 3-14 days or where lower taspine levels are desired, *e.g.*, for pediatric applications. The retentate is recovered from the ultrafilter, can be directly applied or resolubilized in distilled water and then contacted with chromatographic separatory media, preferably ion exchange ("IEC") media and/or solid phase extraction ("SPE") media to remove additional taspine and lower the taspine levels to the desired levels. In one embodiment, the taspine levels are lowered by the additional step of chromatographic separation to below 1% (10,000 ppm) by weight of the resulting enriched concentrate, preferably lowered to below 0.5% (5,000 ppm) by weight of the resulting enriched concentrate. Exemplary resins include ion-exchange type resins, *e.g.*, CM Sepharose sold by Pharmacia Biotech located in Piscataway, NJ; Dowex 50 available from Aldrich located in Milwaukee, WI; WK-10 available from Mitsubishi-Kasei located in White Plains, NY; or absorption type resins, *e.g.*, HP-20 and SP-207 available from Mitsubishi-Kasei. CM Sepharose is the most preferred. Size exclusion resins, *e.g.*, LH-20, sold by Pharmacia Biotech, do not remove taspine in a cost efficient manner. The amount of media required depends upon the type of media used. For example, when using CM-Sepharose, the desired amount of media to treat the filtrate is about 1 part media to 5-20

parts latex to be treated. Preferably for CM-Sepharose, the ratio of media to extract is about 1 part media to about 10 parts latex to be treated. For industrial scale-ups, preferably, the retentate being extracted is contacted in batch with the media, allowed to interact while being stirred for a short period of time to enable the binding of the taspine and other undesired compounds to the separatory media. For example, the resultant slurry can be stirred with the retentate for a period between 15 minutes and 24 hours, preferably about 30 minutes to four hours, most preferably about 2 hours. The resultant slurry can then be filtered to remove the taspine bound media and obtain an enriched proanthocyanidin concentrate having an additionally reduced taspine level. Optionally, the pH of the retentate is adjusted to about the normal pH of the latex, *e.g.*, between 4 to 5, then contacted with the chromatographic media and separated (filtered) as described above. The resulting chromatographed retentate filtrate is then readjusted to neutral, *e.g.*, about pH 7. The concentrate is assayed for taspine to determine the level of taspine remaining after this chromatography step. The chromatography step can be reiterated with fresh media to reduce the taspine levels to the desired levels. Use of this media is distinguished from other chromatographic type resins, *e.g.*, molecular sieve or size exclusion chromatographic resins such as LH-20, which restrict the specific types of proanthocyanidins by molecular weight, which are also ineffective in removing taspine. By utilizing methodologies that have the undesired agent bound to the resin and those methods that need only filtering to remove the undesired material, the need for organic solvents to elute the desired material off of the resin is reduced, lowering the costs of manufacture.

4.1.6 ANTIMICROBIAL AGENT

After the filtrates are separated from the chromatographic media, the resin can be washed with an antimicrobial agent *e.g.*, acetone, to facilitate separation of the desired material from the filter as well as function as an anti-bacterial. In one embodiment, the resin is washed with an amount of 25 to 50 % acetone, preferably about 30% acetone, equal to the resin volume. The acetone wash can be recovered, processed and dried separately from the filtrate, depending upon whether the taspine level is as desired. 10 to 20% acetone, preferably about 15% acetone is added to the filtrate. Additional acetone can be added to raise the acetone level of the filtrate to between 15 and 40 %, preferably about 30% acetone in solution.

4.1.7 DRYING PHASE

In this phase of the preparation, the retentate is dried, to remove residual water and optionally added anti-microbial agent. Preferably the retentate filtrate is dried to a moisture level of 1-20%, more preferably between 3-15% and most preferably between 4-12%. Typically, this drying phase is accelerated by heating. The retentate, essentially free of anti-foaming agents as earlier described, can be dried to the desired moisture level by using a suitable drying method, for example, but not limited to a tray dryer, tumble dryer, drum dryer or cabinet dryer. Other drying methodologies can be used, for example spray drying, evaporative drying under reduced pressure, provided the absence of anti-foaming agents is compensated for. Drying can be achieved in a range from 40° to 60°C, preferably at a temperature of approximately 57°-59°C. Air jets may be directed at the surface of the material to be dried to facilitate the drying.

4.1.8 CHARACTERIZING THE CONCENTRATE

In addition, to determine whether the levels of the taspine, and proanthocyanidins are within the desired limits and provide reproducibility, various assays can be utilized. Assays which can be used include assays to determine the levels present of proanthocyanidins, taspine, phenolics, and moisture content.

4.1.8.1. Ultraviolet Absorbance

One way the useful proanthocyanidin enriched compositions can be detected are by ultraviolet (UV) absorbance. Those skilled in the art will recognize that relevant proanthocyanidin monomers and polymers are colored and typically have broad peaks at about between 190 and 300 nm, for example, at about 190 - 215 nm (205), 225-255 nm (240) and 260-290 nm (275). The relevant fractions can have additional major UV absorption maxima between about 400 nm to about 500 nm, preferably between 425 and 475 nm and most preferably about 460 nm.

4.1.8.2 Phenolic Content

The percentage of total phenolic material in the extract is one way to characterize the extract. Several colorimetric methods for determining the levels of phenolics in the extract were published, including Folin-Dennis and Folin-Ciocalteau methods. Preferably the Folin-Ciocalteau method is used. This assay determines the amount of phenolic material by measuring the visible light absorbance of a sample at about 760 nm and compares the absorbance with that of samples of known concentrations of

Gallic Acid. The phenolic contents in the proanthocyanidin concentrate samples are expressed as weight % Gallic Acid Equivalents (%, w/w, GAE). A range of 50% to 70% of GAE has been observed for the proanthocyanidin concentrate embodiments of the present invention.

4.1.8.3 Proanthocyanidin Percentage

The percentage of proanthocyanidin in the extract is another way to characterize the extract and to provide a reproducible extract. The enriched proanthocyanidin extract can be characterized by a weight percent of at least 35% proanthocyanidin polymer as described in USP 5,211,944 and USP 5,494,661, incorporated by reference herein, present in the extract, preferably at least 40%, more preferably between 50% and 95%. One preferred assay method for determining the amount of this proanthocyanidin polymer in the extract is by utilizing high performance liquid chromatography ("HPLC"). Generally, the chromatogram of a sample of a known amount of the pure proanthocyanidin polymer is passed through an HPLC system is compared with that of a known amount of the isolated sample. The percentage of the pure proanthocyanidin polymer present is determined by comparing the area under peaks appearing at the same retention times. This particular assay is more fully described in the examples, Section 5.1.8.2.

4.1.8.4 Taspine Content

The taspine levels in the extract is another way to characterize the concentrate. The enriched proanthocyanidin extract can be characterized by a weight percent of less than 2% by weight taspine. This level is useful for use as a dietary supplement for administration to humans suffering from traveler's diarrhea or a short term dosage duration of, for example, 0-14 days; or for use in applications were a lower level of taspine is desired, *e.g.*, pediatric applications. The enriched concentrate can be characterized by a weight percent of less than 1% by weight taspine, preferably less than 0.5% by weight. This level is useful for use as a dietary supplement for administration to humans suffering from AIDS related diarrhea and anti-viral for dosage duration's of greater than 14 days. The particular assay preferably used is more fully described in the Examples, Section 5.1.8.3.

4.1.8.5 Moisture Content

The moisture level of the dried extract is another way to characterize the extract and to provide a reproducible extract. The enriched proanthocyanidin extract can be characterized by a moisture content of 1-20%, more preferably between 3-15% and most preferably between 4-12%. While numerous assays are known, the preferred method includes the Karl-Fischer technique (citation), the particular method used is more fully described in the Examples, Section 5.1.8.4.

Other assays known to those of skill in the art can be utilized to characterize the extract prepared according to the methodology of the present invention. Dietary Tannins: Consequences and Remedies, Salunkhe, D.K., et al, eds., CRC Press, pp. 78-111 (1989).

4.1.9 PREFERRED EMBODIMENTS

In a preferred embodiment, the enriched proanthocyanidin polymer extract composition is isolated by the method described below:

Crude Plant Latex *e.g.*, hereinafter CPL) is collected from a *Croton* tree. The CPL is allowed to separate, undisturbed, to allow sediment to settle out to give an aqueous fraction. If the CPL is at room temperature, the CPL is allowed to sit 2 to 8 hours, preferably 2- 4 hours. If the CPL is refrigerated (2-8° C), then the initial setting time is between 2-14 days. If the latex is stored at room temperature, the time allowed to settle is increased, for example 4 hours to 28 days. The resulting liquid supernatant or aqueous soluble fraction of the CPL is carefully removed without disturbing the settled out solid sediment bed. The solid phase sediment is discarded. The resulting aqueous soluble fraction is then mixed with a base (preferably sodium hydroxide). Just before filtration, diatomaceous earth can be added to the latex in the amount of 1-5% by weight of the latex to be processed, to facilitate filtering. The resulting precipitated latex mixture is filtered through diatomaceous earth. The solid residue discarded. The resulting filtrate is concentrated by ultrafiltration through a 1 kilodalton cut off membrane. The retentate is recovered from the microfilter and the permeate is discarded. The retentate, without further modification is contacted with chromatographic media, *e.g.*, an ion-exchange or absorption resin, which removes taspine from the retentate.. After each extraction, the filtrate is retained and tested for taspine levels. If the taspine levels are not within the desired levels, the filtrate is recontacted with the media to lower or reduce the taspine levels to the desired level. The retentate from the ultrafiltration is then concentrated to dryness, for example, using the tray-dryers at approximately 40-57° C (±2° C).

In a specific embodiment, the enriched proanthocyanidin extract characterized by a phenolic content of greater than 45 %, a proanthocyanidin content of greater than 35%, a taspine level of less than 1% by weight, and having a moisture content of less than 12% is prepared as described in Section 5.2, *infra*. In a preferred embodiment, the proanthocyanidin polymer composition is from *Croton lechieri*.

In an alternative embodiment, the proanthocyanidin enriched fraction is preferably obtained by a method comprising the steps of extracting a liquid phase of *Croton* tree latex with a first short chain alcohol; extracting the resulting aqueous retentate from the ultrafiltration step on a solid phase extracting resin; and pooling the fractions eluted from the solid phase extraction resin, *e.g.*, that contain material with detectable ultraviolet absorbance.

The proanthocyanidin enriched extract composition used in the present invention is preferably obtained from a *Croton* spp. They can be obtained using the entire tree or plant, the bark, stems, roots or latex.

In another embodiment of the present invention, the invention comprises an enterically coated matrix, said matrix comprising peppermint oil and a proanthocyanidin enriched extract, wherein the proanthocyanidin enriched extract is obtained from a latex obtained from a *Croton* tree by a method which comprises the steps:

(a) separating latex obtained from a *Croton* tree into a solid phase and a liquid phase;

(b) precipitating the mixed liquid phase from the mixing step with a solvent (*e.g.*, water and isopropyl alcohol);

(c) extracting with n-butanol, a liquid retentate from said liquid phase precipitating step;

(d) concentrating the liquid aqueous retentate from the n-butanol extracting step by ultrafiltration;

(e) extracting an aqueous retentate from said concentrating step on a solid phase extraction resin;

(f) pooling the fractions eluted from the said solid phase extraction resin, *e.g.*, that contain material with UV detectable absorbance; and

a stomach delivery matrix, the stomach delivery matrix comprising ginger and cinnamon.

According to yet another preferred embodiment of the present invention, a novel proanthocyanidin polymer composition can be prepared as follows:

the proanthocyanidin enriched fraction is obtained by obtaining latex from a *Croton* tree. Latex can be obtained from a *Croton* tree by scoring the tree and collecting the

latex accumulating within the scores over a period of time, for example, 24 hours. The latex can be used directly, but is preferably separated into a liquid supernatant phase and a solid sediment phase. One way to effect this separation is to allow the latex to sit undisturbed for a period of time. Another method is to centrifuge the latex, pelleting the solid phase. By whatever method, the resulting liquid phase is carefully removed or siphoned off so as not to disturb the solid phase.

To precipitate the liquid supernatant, to remove additional materials, particularly the sugars, the siphoned liquid phase is precipitated with water. Optionally, the liquid supernatant is precipitated with a first short chain alcohol, *e.g.*, isopropyl alcohol. The ration of siphoned liquid phase to water can range from 25:75 to 50:50, preferably being 1 part siphoned liquid phase to 2 parts water. The ration of isopropyl alcohol to water can range from 10:90 to 90:10, preferably from 40:60 to 60:40 and most preferably 50:50.

The aqueous supernatant resulting from the precipitation step is extracted with a second short chain alcohol, *e.g.*, n-butanol, to additionally purify the aqueous/alcoholic supernatant of undesired organic material. One embodiment contemplated by the inventors includes a biphasic extraction of the aqueous alcoholic supernatant with n-butanol, although any organic solvent which is immersible in water can be used. The supernatant/n-butanol mixture is allowed to sit undisturbed to allow separation.

The aqueous fractions from either the isopropyl alcohol or the n-butanol extraction is then absorbed on a solid phase extraction resin. Exemplary resins include ion-exchange type resins, *e.g.*, Dowex 50 available from Dow Chemical Co., Midland, MI, or absorption type resins, *e.g.*, HP-20 available from Mitsubishi - Kasei, Menlo Park, CA, and SP0207, available from Mitsubishi - Kasei, Menlo Park, CA. Typically, the solution being extracted is loaded onto the resins, washed several times and eluted with a solvent, releasing the desired material from the resin. Use of these resins and the resulting eluted products are distinguished from those eluted from other chromatographic type resins, *e.g.*, molecular sieve or size exclusion chromatographic resins such as LH-20, which restrict the specific types of proanthocyanidins by molecular weight and ar also more expensive. In addition those skilled in the art will recognize that by controlling the process parameters while using solid phase extraction, *i.e.*, eluting solvent, bed volume, flow rate, the pooled fractions can be pre-determined by calculation. Alternatively, those skilled in the art will recognize that the eluting solvent added to a resin batch could constitute a pooled fraction.

In yet another preferred embodiment, the proanthocyanidin enriched extract is isolated by the method described below:

Crude Plant Latex (hereinafter CPL) is collected from a *Croton* tree. The proanthocyanidin enriched extract is isolated from *Croton lechieri*. The CPL is allowed to separate, undisturbed, to allow sediment to settle out to give an aqueous fraction. If the CPL is at room temperature, the CPL is allowed ti sit 6 to 14 days, preferably 7 days. If the CPL is refrigerated (4°C), then the initial setting time is between 36-72 hours, preferably about 48 hours. The resulting liquid supernatant or aqueous soluble fraction of the CPL is carefully removed without disturbing the settled out solid sediment bed. The solid phase sediment is discarded. The resulting aqueous soluble fraction is then precipitated with a solvent selected from water and isopropyl alcohol, preferably with water (Preferably 50% CPL liquid supernatant:50% water to 25% CPL liquid supernatant:75% water, and most preferably 33% CPL liquid supernatant:67% water) and then any insoluble material in the CPL liquid supernatant: water mixture allowed to separate out by settling, *e.g.*, by leaving the mixture at 4°C overnight (12 hours). Optionally, the CPL liquid supernatant is pumped away from the residue, mixed with isopropyl alcohol (IPA) (1:1 V/V) and allowed to settle, *e.g.*, 24 hours at room temperature or 2-8 hours at 4°C. The resulting supernatant is pumped away from the residue, the solid residue discarded. The resulting supernatant is extracted with a short chain alcohol, such as n-butanol, and preferably is extracted multiple times, more preferably three times. After each extraction, the alcohol phase is discarded and the aqueous/alcoholic phase retained. The aqueous/alcoholic phase is concentrated, for example, using an ultrafiltration device with a 1kD cut-off membrane, preferably compatible with short chain alcohols. The purpose of the ultrafiltration is to remove the water from the material. The retentate from the ultrafiltration is then concentrated to dryness, for example, using the tray-dryers at approximately 37°C (± 2°C).

The dried material is subsequently dissolved in water and then extracted on a solid phase extraction resin either by batch or column (for example, Dowex 50, HP-20 or SP-207 resins).

Specifically, the dissolved material is loaded onto a solid phase extraction resin column and then washed with purified water. The proanthocyanidin polymer material is eluted from the solid phase extraction resin with a short chain alcohol (*e.g.*, methanol, ethanol, isopropyl, alcohol and/or acetone. The fractions are collected and monitored with a UV detector, *e.g.*, at a wavelength of 460 nm. Alternatively, the fractions to be pooled can be determined by calculating the fraction size, bed volume and flow rate of the column. Fractions containing the proanthocyanidin enriched extract are combined and concentrated, for example, by ultrafiltration using, *e.g.*, a 1 kD cut-off membrane (as described above for the ultrafiltration step prior to the chromagraphy steps). The retentate may then be concentrated to dryness using a suitable drying method, such as, but not limited to a rotary

evaporator, at a temperature of approximately 37 °C (± 2 C) underreduced pressure. Other suitable drying methodologies include, but are not limited to, tray drying and tumble drying.

The total daily dose of the enriched proanthocyanidin composition is between about 0.5 to 4 gm/day, preferably between about 1 - 3 gm/day, and most preferably about 2.0 gm/day. The total daily dose of the enriched proanthocyanidin composition administered to animals suffering from traveler's diarrhea is between about 0.25 to 2 gm/day, preferably between about 0.5 - 2 gm/day, and most preferably about 1.0 gm/day.

The dosage ranges described generally about, depends on the route and frequency of administration as well as the age, weight and physical condition of the patient. Dietary supplementation and/or treatment can be continued, for example; reduced until the gastrointestinal function is normalized.

4.2 DIETARY SUPPLEMENT FORMULATIONS

The invention provides dietary supplement formulations of an enriched proanthocyanidin polymer extract which protect the extract from the acidity and enzymatic action of gastric secretions. See International Publication WO98/16111. In a preferred embodiment, the dietary supplement formulations of the invention contain the proanthocyanidin polymer enriched concentrate with an enteric coating along with another acceptable vehicle. In another embodiment, the dietary supplement compositions containing the proanthocyanidin polymer composition alternatively include one or more substances that either neutralize stomach acid and/or enzymes or are active to prevent secretion of stomach prepared by methods known in the art, *see, e.g.*, methods described in Remington's Pharmaceutical Sciences, 18th Ed., ed. Alfonso R. Gennaro, Mack Publishing Co., Easton, PA, 1990.

The desired enriched proanthocyanidin polymer concentrate can be provided in any acceptable supplement or form. The dietary supplements can be formulated for oral administration in a matrix as, for example but not limited to, drug powders, crystals, granules, small particles (which include particles sized on the order of micrometers, such as microspheres and microcapsules), particles (which include particles sized on the order of millimeters), beads, microbeads, pellets, pills, microtablets, compressed tablets or tablet triturates, molded tablets or tablet triturates, and in capsules, which are either hard or soft and contain the composition as a powder, particle, bead, solution or suspension. The dietary supplement can also be formulated for oral administration as a solution or suspension in an aqueous liquid, as a liquid incorporated into a gel capsule or as any other convenient formulation for administration, or for rectal administration, as a suppository,

enema or other convenient form. The enriched proanthocyanidin concentrate can also be provided as a controlled release system (see, *e.g.*, Langer, *Science* 249, 1527-1533 (1990)).

The dietary supplement formulation can also include any type of acceptable excipients, additives or vehicles. For example, but not by way of limitation, diluents or fillers, such as dextrates, dicalcium phosphate, calcium sulfate, lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch, sorbitol, sucrose, inositol, powdered sugar, bentonite, microcrystalline cellulose, or hydroxypropylmethylcellulose may be added to the proanthocyanidin polymer composition to increase the bulk of the composition. Also, binders, such as, but not limited to, starch, gelatin, sucrose, glucose, dextrose, molasses, lactose, acacia gum, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapgol husks, carboxymethylcellulose, methylcellulose, polyvinylpyrrolidone, Veegum and starch arabogalactan, polyethylene glycol, ethylcellulose, glyceryl monostearate and waxes, may be added to the formulation to increase its cohesive qualities. Additionally, lubricants, such as, but not limited to, glyceryl monostereate, talc, magnesium stearate, calcium stearate, stearic acid, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, carbowax, sodium lauryl sulfate, and magnesium lauryl sulfate may be added to the formulation. Also, glidants, such as but not limited to, colloidal silicon dioxide, magnesium silicate or talc may be added to improve the flow characteristics of a powdered formulation. Finally, disintegrants, such as but not limited to, starches, clays, celluloses, algins, gums, crosslinked polymers (*e.g.*, croscarmelose, crospovidone, and sodium starch glycolate), Veegum, methylcellulose, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, alginic acid, guar gum, citrus pulp, carboxymethylcellulose, or sodium lauryl sulfate with starch may also be added to facilitate disintegration of the formulation in the stomach or intestine.

In another preferred embodiment of the invention, the enriched proanthocyanidin polymer concentrate and any other component for delivery to the colon is formulated with a substance that protects the proanthocyanidin polymer® composition from the stomach environment. In a more preferred embodiment, the proanthocyanidin composition is enteric coated. Enteric coatings are those coatings that remain intact in the stomach, but will dissolve and release the contents of the dosage form once it reaches the small intestine. A large number of enteric coatings are prepared with ingredients that have acidic groups such that, at the very low pH present in the stomach, *i.e.* pH 1.5 to 2.5, the acidic groups are not ionized and the coating remains in an undissociated, insoluble form. At higher pH levels, such as in the environment of the intestine, the enteric coating is converted to an ionized form, which can be dissolved to release the proanthocyanidin polymer concentrate. Other enteric coatings remain intact until they are degraded by

enzymes in the small intestine, and others break apart after a defined exposure to moisture, such that the coatings remain intact until after passage into the small intestines.

Polymers which are useful for the preparation of enteric coatings include, but are not limited to, shellac, starch and amylose acetate phthalates, styrine-maleic acid copolymers, cellulose acetate succinate, cellulose acetate phthalate (CAP), polyvinylacetate phthalate (PVAP), hydroxypropylmethylcellulose phthalate (grades HP-50 and HP-55), ethylcellulose, fats, butyl stearate and methacrylic acid-methacrylic acid ester copolymers with acid ionizable groups ("EUDRAGIT™"), such as "EUDRAGIT™ L 30D", "EUDRAGIT™ L 100-55", and "EUDRAGIT™ L 30D-55". In a preferred embodiment, the pharmaceutical composition contains a proanthocyanidin polymeric extract and the enteric coating polymer "EUDRAGIT™ L 30D-55", an anionic copolymer of methacrylic acid and methyl acrylate with a mean molecular weight of 250,000 Daltons.

The disintegration of the enteric coating occurs either by hydrolysis by intestinal enzymes or by emulsification and dispersion by bile salts, depending upon the type of coating used. For example, esterases hydrolyze esterbutyl stearate to butanol and stearic acid and, as the butanol dissolves, the stearic acid flakes off of the medicament. Additionally, bile salts emulsify and disperse ethylcellulose, hydroxypropyl-methylcellulose, fats and fatty derivatives. Other types of coatings are removed depending on the time of contact with moisture, for example coatings prepared from powdered carnauba wax, stearic acid, and vegetable fibers of agar and elm bark rupture after the vegetable fibers absorb moisture and swell. The time required for disintegration depends upon the thickness of the coating and the ratio of vegetable fibers to wax.

Application of the enteric coating to the proanthocyanidin polymer composition or a matrix containing such can be accomplished by any method known in the art for applying enteric coatings. For example, but not by way of limitation, the enteric polymers can be applied using water. Some other enteric polymers, such as methacrylic acid-methacrylic acid ester copolymers can also be applied using water as a dispersant. The volatility of the solvent system must be tailored to prevent sticking due to tackiness and to prevent high porosity of the coating due to premature spray drying or precipitation of the polymer as the solvent evaporates.

Furthermore, plasticizers can be added to the enteric coating to prevent cracking of the coating film. Suitable plasticizers include the low molecular weight phthalate esters, such as diethyl phthalate, acetylated monoglycerides, triethyl citrate, polyethyl glycoltributyl citrate, triethyl acetates and triacetin. Generally, plasticizers are added at a concentration of 5% to 10% by weight of enteric coating polymer weight. Other additives such as emulsifiers, for example detergents and simethiconel, and powders, for

example talc, may be added to the coating to improve the strength and smoothness of the coating. Additionally, pigments may be added to the coating to add color to the dietary supplement formulation.

In additional embodiments, the dietary supplements of the enriched proanthocyanidin polymer extracts are provided as enteric coated beads in hard-shell gelatin capsules or as another dissolvable layer coating the capsule. Gelatin capsules are preferred when the enriched proanthocyanidin extract is in the form of a suspension, gel or liquid

In general, the enriched proanthocyanidin polymer extract granules and powder can be prepared using any method known in the art, such as but not limited to, crystallization, spray-drying or any method of comminution, preferably using a high speed mixer/granulator. Examples of high speed mixer/granulators include the "LITTLEFORD LODIGE™" mixer, the "LITTLEFORD LODIGE™" MGT mixer/granulator, and the "GRAL™" mixer/granulator. During the high-shear powder mixing, solutions of granulating agents, called binders, are sprayed onto the powder to cause the powder particles to agglomerate, thus forming larger particles or granules. Granulating agents which are useful for preparing the proanthocyanidin polymer composition granules, include but are not limited to, cellulose derivatives (including carboxymethylcellulose, methylcellulose, and ethylcellulose), gelatin, glucose, polyvinylpyrrolidone (PVP), starch paste, sorbitol, sucrose, dextrose, molasses, lactose, acacia gum, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, Veegum and larch arabogalactan, polyethylene glycol, and waxes. Granulating agents may be added in concentrations ranging from 1 to 30% of the mass of the particles or granules..

The proanthocyanidin polymer composition powder or granules are preferably coated using the fluidized bed equipment. The granules or powder may then be covered with a seal coat of Opadry Clear (mixed with water). A preferred enteric coating for the proanthocyanidin polymer composition is EUDRAGIT™ L 30D-55" applied as an aqueous dispersion containing 30% w/w dry polymer substance, to which plasticizers, *e.g.*, triethyl citrate, are added to improve the elasticity of the coating, and talc is added to reduce the tendency of the enteric coating polymer to agglutinate during the application process and to increase the smoothness of the film coating. An example of the final composition of the enteric coated tablet (648 mg [100 w/w]) is 600 mg proanthocyanidin concentrate (92.6% w/w); 35.5 mg (5.5% w/w); 3.6 mg (0.6% w/w) triethyl citratel and 8.9 mg (1.3% w/w) talc. One embodiment of the present invention is described in Example 5.2.

The enteric coated proanthocyanidin polymer composition granules or powder particles can further be suspended in a solution for oral administration, particularly for pediatric administration. The suspension can be prepared from aqueous solutions to

which thickeners and protective colloids are added to increase the viscosity of the solution to prevent rapid sedimentation of the coated powder particles or granules. Any material which increases the strength of the hydration layer formed around suspended particles through molecular interactions and which is pharmaceutically compatible with the proanthocyanidin polymer composition can be used as a thickener, such as but not limited to, gelatin, natural gums (*e.g.*, tragacanth, xanthan, guar, acacia, panwar, ghatti, etc.), and cellulose derivatives (*e.g.*, sodium carboxymethylcellulose, hydroxypropyl-cellulose, and hydroxypropylmethylcellulose, etc.). Optionally, a surfactant such as Tween may be added to improve the action of the thickening agent. A preferred suspension solution is a 2% w/w hydroxypropylmethylcellulose solution in water containing 0.2% Tween.

The proanthocyanidin polymer composition can also be formulated as enteric coated tablets. In a preferred embodiment, the proanthocyanidin polymer composition is granulated with any pharmaceutically acceptable diluent (such as those listed above) by the methods described above for preparing the proanthocyanidin polymer composition granules. Then, the granules are compressed into tablets using any method well known in the art, for example but not limited to, the wet granulation method, the dry granulation method or the direct compression method. Preferred diluents include, but are not limited to, microcrystalline cellulose ("AVICEL™ M 200") and dextrates ("EMDEX™"). Additionally, disintegrants, such as those described above, and lubricants, such those above, may also be added to the tablet formulation.

An embodiment of the present invention can include a core tablet including 40-99.5% proanthocyanidin concentrate, 0-5% glidant, 0.5-10% lubricant, 0-10% disintegrant with the balance being filler (0-59.5%). A preferred embodiment core tablet formulation can include 0.25%-1% glidant; 2-5% lubricant, 5-10% disintegrant, and the balance filler (0.05 to 59%). A preferred core tablet formulation is a core tablet of 60-78 % proanthocyanidin concentrate, 0.25%-1% colloidal silicon dioxide; 2-5% glyceryl monostereate; 5-10% sodium starch glycolate and the balance (5.75 -84 %) microcrystalline cellulose (Avicelll M 200). One example of a per tablet core tablet formulation as more fully described in Section 5.2 contains 350 mg or more, *e.g.*, 400 mg of the enriched proanthocyanidin polymer concentrate (67% w/w), 21 mg of the lubricant glyceryl monostearate (3.5% w/w), 3 mg of colloidal silicon dioxide (0.5% w/w), and 48 mg of the disintegrant, sodium starch glycolate (8.0 % w/w) and the weight of microcrystalline cellulose ("AVICEL™ M 200") necessary to bring the mixture up to 600 mg (128 mg or 21.3% w/w).

The core tablet can be enterically coated with a formulation of 50%-80% enteric coated polymer, preferably 0.05 - 5% plasticizer and the balance, *e.g.*, 15-49.95%

filler. A more preferred embodiment is a coating formulation of 60-78% copolymer; 0.05-1% plasticizer; and 12.5-39.95 % filler. A most preferred embodiment is more fully described in Section 5.2. The tablets are coated with an enteric coating mixture prepared from quantities dependent upon the amount of tablets to be coated. For example, for about 100 kg quantity of core tablets, each tablet was coated with 35.5 mg EUDRAGIT™ L 30 D-55, 3.6 mg triethyl citrate, and 8.9 mg talc, dry weight after drying. This formulation may be prepared by any method well known in the art or by the method described in Section 5.2, *infra*.

The proanthocyanidin polymer composition formed into small particles (which include particles sized on the order of micrometers, such as microspheres and microcapsules), particles (which include particles sized on the order of millimeters), drug crystals, pellets, pills and microbeads can be coated using a fluidized-bed process. This process uses fluidized-bed equipment, such as that supplied by "GLATT™", "AEROMATIC™", "WURSTER™", or others, by which the proanthocyanidin polymer composition cores are whirled up in a closed cylindrical vessel by a stream of air, introduced from below, and the enteric coat is formed by spray drying it onto the cores during the fluidization time. To coat tablets or capsules, Accela-Cota coating equipment ("MANESTY™") can be used. By this process, the tablets or capsules are placed in a rotating cylindrical coating pan with a perforated jacket and spraying units are installed within the pan and the dry air is drawn in through the rotating tablets or capsules. Any other type of coating pan, such as the "COMPU-LAB™" pan, Hi-coates "GLATT™" immersion sword process, the "DRIAM™" Dricoater, "STEINBERG™" equipment, "PELLEGRINI™" equipment, or "WALTHER™" equipment can also be used.

In another embodiment, the proanthocyanidin polymer composition is formulated with a compound or compounds which neutralize stomach acid. Alternatively, the dietary supplement composition containing the proanthocyanidin polymer composition is administered either concurrent with or subsequent to administration of a dietary supplement composition which neutralize stomach acid. Compounds, such as antacids, which are useful for neutralizing stomach acid include, but are not limited to, aluminum carbonate, aluminum hydroxide, bismuth subnitrate, bismuth subsalicylate, calcium carbonate, dihydroxyaluminum sodium carbonate, magaldrate, magnesium carbonate, magnesium hydroxide, magnesium oxide, and mixtures thereof.

In another embodiment, the proanthocyanidin polymer composition is administered with a substance that inactivates or inhibits the action of stomach enzymes, such as pepsin. Alternatively, the dietary supplement composition containing the proanthocyanidin polymer composition is administered either concurrent with or subsequent

to administration of a dietary supplement composition active agent to inactivate or inhibit the action of stomach enzymes. For example, but not by way of limitation, protease inhibitors, such as aprotin, can be used to inactivate stomach enzymes.

In another embodiment, the proanthocyanidin polymer composition is formulated with a compound or compounds which inhibit the secretion of stomach acid. Alternatively, the dietary supplement composition containing the proanthocyanidin polymer composition is administered either concurrent with or subsequent to administration of a dietary supplement composition active to inhibit the secretion of stomach acid. Compounds which are useful for inhibiting the secretion of stomach acid include, but are not limited to, ranitidine, nizatidine, famotidine, cimetidinel and misoprostol.

4.2.1 ADDITIONAL HERBAL AGENTS

As indicated above, according to another alternative embodiment of the invention, the dietary supplements comprise an enriched proanthocyanidin polymer composition as described above in Section 4.2 together with one or more herbal agents as described below.

4.2.1.1 PREPARATION OF GINGER

Ginger can be obtained from the root (rhizome) of the plant. Such roots can be obtained from various commercial sources. The plant material can be shredded, ground, macerated, chopped, pounded or otherwise treated prior to use. The ginger used is preferably ginger powder of the local pharmacoperal standard (*e.g.*, Society of Japan Pharmacopeia, The Pharmacopeia of Japan, Hirokawa Publishing Co., Tokyo, Japan (1976). For the purpose of this invention, the dietary supplement includes a total daily dose of 1-5 gm of ginger powder, for example about 3 X (.350-1) gm. See, PDR for Herbal Medicines, pp. 1230 (1998), O'Hara, M.A., et al., pp. 530-531 (1996).

4.2.1.2 PREPARATION OF CINNAMON

Cinnamon (*Cinnamonum aromaticum* and *C. verum*) suitable for the present invention, can be purchased from various commercial sources. For example, bark peeled from 2-3 inch thick branches can be separated from the cork and other rind and dried in the sun for about 24 hours. PDR for Herbal Medicines, pp. 750 (1998). See also powdered cinnamon (Cinamoni Cortex Pulveratus; Nippon Furimatsu Yakuhin & Co., Osaka, japan). The cinnamon used is preferably cinnamon powder of the local pharmacoperal standard (*e.g.*, Society of Japan Pharmacopeia. The Pharmacopeia of Japan. Hirokawa Publishing Co., Tokyo, Japan (1976)). If this herbal agent is in the form of cinnamon powder, the

dietary supplement includes a daily dose of about -14 g/day, for example 3 X (.35 - 1.0 g)/day. If this herbal agent is in the form of cinnamon essential oil, the dosage is about 0.05 to 0.2 gm, PDR for Herbal Medicines, pp. 750 (1998). Cinnamon, as with ginger, should be formulated for delivery to the stomach.

4.2.1.3 PREPARATION OF PEPPERMINT OIL

Peppermint (*Metha piperita*) oil suitable for the present invention can be purchased from various commercial sources (for example Tillotts Laboratories, United Kingdom). The preferred form of peppermint is peppermint oil. The oil is generally extracted from the aerial parts of the flowering plant, the dried leaves and flowering branch tips, the fresh flowering plant and the whole plant. PDR for Herbal Medicines, pp. 971 (1998). For the purposes of this invention, the daily dose of peppermint oil is between 0.4-0.8 ml/day, preferably about 0.6 ml/day. The amount per single dose depends upon the number of doses per day, *e.g.*, 3. Peppermint oil should be formulated for delivery to the colon, *e.g.*, enterically coated.

The dosage ranges described generally above, depends on the route and frequency of administration as well as the age, weight and physical condition of the patient. Dietary supplementation and/or treatment can be continued, for example, reduced until the gastrointestinal distress is alleviated.

4.2.1.4 WEIGHT RATIOS

The weight ratio of the proanthocyanidin enriched extract to the at least one additional herbal agent is about 0.333 to 1.0 parts proanthocyanidin enriched extract to 1.0 part herbal agent, *e.g.*, ginger and cinnamon. The weight ratio of the proanthocyanidin enriched extract to peppermint oil is in a weight ratio of about 0.5 to 1.5 parts proanthocyanidin enriched extract to about 1 part peppermint oil. The preferable weight ratios are those compliant with the respective daily dosage requirements. See Section 4.6. For example, the weight ratios of the daily dosages should be between 0.5-2.0 g/day of proanthocyanidin enriched extract, 1-4 g/day of ginger, 1-4 g/day of cinnamon, and 0.4-0.8 and preferably 0.6 ml (mg)/day of peppermint oil. One example weight ration falling within the daily dosage ranges, is 500-100 mg proanthocyanidin enriched extract, 1g of ginger, 1g of cinnamon and 0.6 ml (approximately 600 mg) of peppermint oil. Another example weight ratio falling within the daily dosage ranges is 25 parts proanthocyanidin enriched extract, 35 parts ginger, 35 parts cinnamon and 20 parts peppermint oil. Still another example of weight ratios is described in Section 4.6 as 295 mg proanthocyanidin enriched extract, 413 mg cinnamon powder, and 413 mg ginger powder.

4.2.1.5 PHARMACEUTICAL FORMULATIONS

The invention provides pharmaceutical formulations of a proanthocyanidin enriched extract which protect the extract from the acidity and enzymatic action of gastric secretions, *e.g.*, stomach acid and pepsin, for delayed delivery to the colon. See International Publication WO98/16111 (1998). In a preferred embodiment, the dietary supplement formulations of the invention contain the proanthocyanidin polymer enriched extract and any other herbal agent desired to be delivered to the colon, *e.g.*, peppermint oil, with an enteric coating along with another pharmaceutically acceptable vehicle. The dietary supplement formulations also contain at least one additional herbal agent formulated for delivery to the stomach. Formulated for delivery to the stomach entrails the formulation being dissolving coatings matrix or layer. Fast dissolving means that the matrix begins dissolving within minutes and is substantially all dissolved within 2 hours, *e.g.*, the average item for stomach emptying. Those skilled in the art will recognize that the time of dissolving can be varied by altering the actual mixture of materials in the coating matrix or layer and their relative solubility in differing pHs. The additional herbal agents can be contained in a matrix for delivery to the stomach. In another embodiment, the pharmaceutical compositions containing the proanthocyanidin polymer composition alternatively include one or more substances that either neutralize stomach acid and/or enzymes or are active to prevent secretion of stomach prepared by methods known in the art, *see, e.g.*, methods described in Remington's Pharmaceutical Sciences, 18th Ed., ed. alfonso R. Gennaro, Mack Publishing Co., Easton, PA, 1990.

The desired herbal agents, including the proanthocyanidin enriched extract can be provided in any therapeutically acceptable supplement or pharmaceutical form. The dietary supplements can be formulated for oral administration in a matrix as, for example but not limited to, drug powders, crystals, granules, small particles (which include particles sized on the order of micrometers, such as microspheres and microcapsules), particles (which include particles sized on the order of millimeters), beads microbeads, pellets, pills, microtablets, compressed tablets or tablet triturates, molded tablets or tablet triturates, and in capsules, which are either hard or soft and contain the proanthocyanidin enriched extract and other herbal agents as a powder, particle, bead, solution or suspension. The dietary composition can also be formulated for oral administration as a solution or suspension in an aqueous liquid, as a liquid incorporated into a gel capsule or as any other convenient formulation for administration, or for rectal form. The enriched proanthocyanidin extract can also be provided as a controlled release system (see, *e.g.*, Langer, 19990, *Science* 249:1527-1533).

In addition, since the proanthocyanidin enriched extract and peppermint oil are preferably formulated for delivery to the colon, these agents can be formulated in a first matrix while the other at least one additional herbal agents can be formulated in a second matrix formulated for delivery to the stomach. The pharmaceutical compositions containing a proanthocyanidin polymer and another herbal agent can also be formulated as described above in Section 4.2 hereof.

Another proximal colonic delivery carrier of this invention is a pulse capsule, such as Pulsincap.RTM. As used herein, "pulse capsules" include capsules described in U.K. Patent Application Nos. 2,230,441A and 2,230,442A of National Research Development Corporation, published Oct. 24, 1990; and PCT Patent Application No. WO91/12795 of National Research Corporation, published Sep. 5, 1991, all of which have U.S. patent application equivalents and are incorporated herein by reference. One form of such a capsule is Pulsincap.TRM. manufactured by Scherer DDS, Clydesbanke, Scotland, U.K. Examples of pulse capsules comprise a water-insoluble male capsule shell, a water-dispersible or swellable hydrophilic plug, and a water-soluble male capsule shell, a water-dispersible or swellable hydrophilic plug, and a water-soluble female capsule shell. The male and female shells preferably have the size, shape, and fit of conventional hard gelatin capsule male and female mating shells. For preferred pulse capsule unit dosage form compositions of this invention, the proanthocyanidin extract containing matrix is contained in the male capsule shell and enclosed with the hydrophilic plug such that the hydrophilic plug blocks the entire opening of the male shell. The female shell covers the exposed portion of the plug and extends along the outer cylindrical surface of the male shell. In contact with the fluids of the stomach and the intestines beyond, the female shell of a pulse capsule dissolves and the hydrophilic plug hydrates. The composition and size of the hydrophilic plug is selected such that the hydrophilic plug will disengage from the male capsule shell after a predetermined amount of time, releasing the proanthocyanidin extract containing matrix at the approximate time when the dosage form reaches the colon. A preferred pulse capsule proximal colonic delivery carrier additionally comprises a pH sensitive material that will dissolve at a pH typically associated with the upper small intestine (duodenum). This coating encompasses the capsule such that the female capsule shell does not dissolve, and hydration of the hydrophilic plug does not begin until the unit dosage form has emptied from the stomach. This controlled delay eliminates variability due to differences in gastric emptying time (time between ingestion of the unit dosage form and its being emptied from the stomach) when determining the amount of time desired between dissolution of the female shell and disengagement of the plug from the male shell opening. A preferred composition of this invention include the incorporation of proanthocyanidin

enriched matrix into a Pulsincap.RTM capsule onto which an enteric coating of the type described in the preceding paragraph is applied.

The dietary supplements of this invention can optionally include herbal ingredients in addition to the enriched proanthocyanidin. non-limiting examples of other active drug agents and amounts typically present in such dietary supplements include the following: proanthocyanidin enriched extract 295 mg; cinnamon powder 413 mg; ginger powder 413 mg; ducosate sodium 250 mg; lubricant 4 mg; disintegrant 45 mg and glidant 78 mg; about 1500 mg total (3 X 500 mg tablets).

4.3 APPLICATIONS OR METHODS OF USE

The dietary supplement formulations and methods of the invention are useful as dietary supplements in promoting normal or healthy function of the gastrointestinal tract. They are also useful in administering to mammals suffering from the symptoms of gastrointestinal disorders, particularly symptoms of irritable bowel syndrome.

The dietary supplement formulations of the proanthocyanidin polymer composition can be used administered to mammals suffering from any type of gastrointestinal disorders in either humans or animals.

In another embodiment, the dietary supplement formulation is administered to mammals suffering from secretory diarrhea caused by non-infectious etiologies, such as but not limited to, non-specific diarrhea, inflammatory bowel syndrome.

In another embodiment, the dietary supplement formulations of the invention are useful as dietary supplements for administration to those afflicted with HIV-Associated Chronic Diarrhea in patients with AIDS. In yet another embodiment, the dietary supplement formulation is useful as dietary supplements for administering to infants or children suffering from diarrhea, including but not limited to, diarrhea caused by rotavirus.

The dietary supplement formulations of the invention can also be useful as dietary supplements in non-human animals, particularly in farm animals, such as but not limited to, bovine animals, swine, ovine animals, poultry (such as chickens), and equine animals, and other domesticated animals such as canine animals and feline animals. In particular the dietary supplement formulations of the invention can be useful as dietary supplements in non-human animals, particularly food animals such as cattle, sheep and swine, suffering from diarrhea caused by bacterial pathogens such as enterotoxigenic, enterohemorrhagic and other *E. coli*, *Salmonella* spp., *Clostridium perfringens*, *Bacteriodes fragilis*, *Campylobacter* spp., and *Yersinia enterocolitica*, protozoal pathogens, particularly *Cryptosporidium parvum*, and viral agents, particularly rotaviruses and coronaviruses, but also togavirus, parvovirus, calicivirus, adenoviruses, bredaviruses, and astroviruses.

Another embodiment includes when cinnamon is employed as part of the preparation, gastritis and gastric ulcers are included as a therapeutic option.

Additionally, the dietary supplement formulations of the invention may also be administered as a dietary supplement to humans and non-human animals. By way of example, but not by way of limitation, a proanthocyanidin polymer composition dietary supplement formulation can be administered to tourists traveling to a country where there is a risk of traveler's diarrhea at a time or times that are effective for anticipating the disease. The dietary supplement compositions of the invention can be taken as a dietary supplement by AIDS patients in anticipation of the occurrence of HIV-Associated Chronic Diarrhea. Also, the dietary supplement compositions of the invention can be administered as a dietary supplement to children in a community threatened with cholera epidemic or rotavirus epidemic. Likewise, the dietary supplement compositions of the invention can be administered to farm animals, particularly young or recently weaned farm animals, in anticipation of the development of diarrheal disease.

When used according to the formulations and methods of the present invention in anticipation of secretory diarrhea, recommended daily dosage ranges of the dietary supplement formulations of the proanthocyanidin polymer composition for oral administration are in the range of 1 to 4 gm per day, preferably about 1 to 3 gm per day, and also optionally 2 gm per day. It should be appreciated that the appropriate dose will depend upon the type and severity of the secretory diarrhea. It has been found that human subjects can tolerate at least up to 2 grams of the proanthocyanidin polymer composition per day (25-30 mg/kg/day) for up to 2 days. It is believed that doses may exceed 40 mg/kg per day, optionally up to 100 mg/kg per day, if such dosages are necessary to treat the secretory diarrhea.

When used according to the formulations and methods of the present invention as a prophylaxis for secretory diarrhea, effective dosage ranges of the dietary supplement formulations of the proanthocyanidin polymer composition for oral administration are in the range of 0.1 to 100 mg/kg per day, preferably about 0.1 to about 40 mg/kg per day, optionally 0.1 to 25 mg/kg per day, and also optionally 0.1 to 10 mg/kg per day. It should be appreciated that the appropriate dose will depend upon the type and severity of the secretory diarrhea to be prevented.

When used according to the formulations and methods of the present invention in anticipation of traveler's diarrhea, effective daily dosage ranges of the dietary supplement formulations of the proanthocyanidin polymer composition for oral administration are in the range of 0.5 to 2 gm per day, preferably about 0.5 to 1.5 gm per day, and also optionally 1.0 gm per day. In another embodiment the daily dosage may be in

the form of 7.5 - 15 drops of the active concentrate. It should be appreciated that the appropriate dose will depend upon the type and severity of the secretory diarrhea. It is believed that doses may exceed 20 mg/kg per day, optionally up to 50 mg/kg per day, if such dosages are necessary to treat the traveler's diarrhea.

When used according to the formulations and methods of the present invention as a prophylaxis for secretory diarrhea, effective dosage ranges of the dietary supplement formulations of the proanthocyanidin polymer composition for oral administration are in the range of 0.1 to 100 mg/kg per day, preferably about 0.1 to about 40 mg/kg per day, optionally 0.1 to 25 mg/kg per day, and also optionally 0.1 to 10 mg/kg per day. It should be appreciated that the appropriate dose will depend upon the type and severity of the secretory diarrhea to be prevented. The proanthocyanidin polymer composition can be administered to mammals suffering from secretory diarrhea in any acceptable form. The dietary supplement composition can be administered orally, in the form of, such as but not limited to, drug crystals, granules, small particles (which include particles sized on the order of micrometers, such as microspheres and microcapsules), particles (which include particles sized on the order of millimeters) beads, microbeads, pellets, pills, microtablets, compressed tablets or tablet triturates, molded tablets or tablet triturates, and in capsules, which are either hard or soft and contain the composition as a powder, particle, bead, solution or suspension. The dietary supplement composition can also be administered orally, as a solution or suspension in an aqueous liquid, as a liquid incorporated into a gel capsule or as any other convenient formulation for administration, or rectally, as a suppository, enema or other convenient form.

In a preferred embodiment, an enteric coated dietary supplement composition containing the proanthocyanidin polymer composition is administered to mammals suffering from secretory diarrhea. In a more preferred embodiment, the enteric coated dietary supplement composition are enteric coated tablets, optionally containing other excipients such as colloidal silicon dioxide, a glidant; microcrystalline cellulose, a filler and glyceryl monostereate, a dispersant. In another embodiment, a dietary supplement composition containing the proanthocyanidin polymer concentrate and a compound which neutralizes stomach acid or inhibits the secretion of stomach acid is administered for the treatment of secretory diarrhea. In yet another embodiment, a dietary supplement composition containing the proanthocyanidin polymer composition is administered either concurrent with or subsequent to administration of a pharmaceutical composition which either neutralizes stomach acid or inhibits the secretion of stomach acid for treatment of secretory diarrhea. The proanthocyanidin polymer composition can also be formulated as a suppository for rectal administration.

The dietary supplement formulations of the invention can also be administered either alone or in combination with other agents for treatment or amelioration of secretory diarrhea symptoms such as rehydration agents, antibiotics, anti-motility agents, and fluid adsorbents, such as attapulgite.

The dietary supplement formulations of the invention can also be incorporated into animal feed for use in treating secretory diarrhea in animals such as bovine animals, swine, ovine animals, poultry, equine animals, canine animals, and feline animals.

When used according to the formulations and methods of another embodiment of the present invention as a dietary supplement, effective daily dosage ranges included within the dietary supplement include a proanthocyanidin enriched extract in the range of 0.5 to 2 gm of per day, with a single dosage (for 3X per day) of about 0.25 to 0.5 g of the proanthocyanidin enriched extract; ginger powder in the range of 1-4 g per day, with a single dosage (for 3X per day) of about 0.35 to 1.0 g of ginger powder; cinnamon powder in the range of 1-4 g per day, with a single dosage (for 3X per day) of about 0.35 to 1.0 g of cinnamon powder; peppermint oil in the range of about 0.5-0.7 ml (mg) per day preferably about 0.6 ml (600 mg) per day, with a single dosage (for 3X per day) of about 0.2 ml (200 mg) of peppermint oil for oral administration. It should be appreciated that the appropriate dose will depend upon the type and severity of the gastrointestinal distress.

The following series of Examples are presented for purposes of illustration and not by way of limitation on the scope of the invention.

5. EXAMPLES: PREPARATION OF DIETARY SUPPLEMENT FORMULATIONS

Described below are illustrative methods for the manufacture and packaging for different preferred pharmaceutical formulations of the proanthocyanidin polymer composition from *C. lechlieri* according to the present invention.

5.1. EXAMPLE: ENRICHED PROANTHOCYANIDIN POLYMER CONCENTRATE

In one series of experiments, a novel enriched proanthocyanidin extract having a taspine level of less than 0.5% and having a proanthocyanidin level of about 40% (used to prepare the formulations in Examples 8.5 and 8.6 above) was isolated from the latex of the *Croton lechleri* plant as follows:

5.1.1 THE LATEX

C. Lechleri trees were tapped and felled near the village of San Pablo de Cuyana on the Nana River, 100 kilometers from Iquitos, Peru. The latex was obtained over a period of 24 hours by scoring the trees.

5.1.2 PRECIPITATION PHASE

The latex obtained from the Croton lechleri trees was (1800 kilograms) was transferred from the shipping container with a low shear transfer pump and dedicated tubing to a 500 gallon reactor. Care was taken to minimize the amount of sediment transferred from the container to the reactor. The pH of the combined raw latex mixture within the reactor was recorded. The pH of the latex was adjusted to pH 7.0 with about 56 kg of 2.5 N NaOH. by the addition of 2.5 N NaOH in small increments so as not to exceed pH 7.0. The mixture was cooled in the reactor to room temperature. The pH adjustment of the batch required about 1 hour.

5.1.3 FILTERING PHASE

16 kg of diatomaceous earth (CELATOM FW-6 ™ brand diatomaceous earth sold by Great Western Chemical of Richmond CA.) was mixed with purified water until a smooth flowing slurry was formed. The slurry was transferred onto a nutsch (48 inch diameter, filtering surface of about 1.17 square meters) to form a 1 inch bed. 90 kg of the diatomaceous earth, 5% by weight of the latex charge (1800 kg) was added to the batch and mixed for 10 minutes. The diatomaceous earth bed was washed with water until the filtrate was clear. The pH adjusted mixture from the reactor was transferred evenly across the top to the diatomaceous earth bed. The solution was allowed to percolate through the diatomaceous earth bed by gravity for two minutes before applying a vacuum. When the color of the filtrate turned a reddish brown, the filtrate was collected. An initial 45.8 kg portion of the reactor mix was pumped onto the bed to displace the water in the bed, 22.6 kg of filtrate were collected when the reactor was emptied. The solid content analysis revealed 31.6 gm solid in solution. This initial filtrate was discarded. Filtration continued by pumping 100 and 230 kg aliquots of the reactor mix onto the diatomaceous earth bed. A total of 302 gallons (1144 kg) of filtrate was collected. The precipitated residue was retained until the entire contents was filtered.

5.1.4 CONCENTRATING PHASE

The resulting filtrate was concentrated by passing (ultrafiltering) the resulting filtrate through a 1 kd cellulose-based membrane . The final weight of the concentrate was targeted to be about 360 kg. The retentate was 436 kg and the rest was permeate. The solid content of the retentate was estimated to be about 98 kg. A sample was removed, dried and weighed to provide the estimate. The pH of the retentate was adjusted to 4.0 with 27 kg of 10% hydrochloric acid solution. The total weight of the pH adjusted retentate was 463 kg solid content. The filtrate was placed in a 2° - 8° storage facility overnight. The filtrate from the diatomaceous earth filtering step was allowed to equilibrate at room temperature.

5.1.5 REMOVING ADDITIONAL TASPINE PHASE

The retentate from the ultrafiltering step was assayed to 1 upon pH adjustment indicated a taspine level of 19,342 and 20,000 by the assay of Sections 5.1.8.3.1 or 5.1.8.3.2 depending on whether the sample was solid or liquid. The retentate was warmed to room temperature by passing through a heat exchanger. 180 kg of CM-Sepharose was mixed with 463 kg of the retentate. A 30 minute sample of the reactor mix of filtrate and CM-Sepharose was analyzed and indicated to have a taspine level of 7,466 ppm, and 8,157 ppm by the assay of Sections 5.1.8.3.1 or 5.1.8.3.2. The two hour sample exhibited a taspine level of 7,522 ppm. The batch was sampled for taspine levels at t = 0, 2, and 4 hours. A second solid phase extraction procedure was repeated with the sample to reduce the level of taspine present in the extracts. A second batch was contacted with 150 kg of regenerated resin. The taspine assay indicated that after 30 minute from the time the filtrate was contacted with the chromatographic resin, the taspine level was about 3280 ppm. When the taspine levels reached this level, i.e. 3280 ppm or 0.328%, the batch was filtered to separate the resin from the filtrate. This provided a dry weight yield of 38 kg.

5.1.6 ANTI-MICROBIAL AGENT

An antimicrobial agent was added to reduce microbial content of the concentrate and to increase the yield. Accordingly, the resin remaining on the nutche after the filtering was washed with acetone to remove any residual materials remaining on the resin. An amount of 30% acetone equal to twice the volume of resin used was prepared. After the first contact with the CM-Sepharose, taspine bound resin was washed, filtered, recovering 495 kg of resin wash. The wash was assayed for taspine levels. The filtrate was adjusted to pH 7.0 with 2.5 N Sodium Hydroxide solution. Sufficient 30% acetate was

added to the filtrate (86 L), to bring the final concentration to 21% acetone in the solution. (575 L). The 30% acetone had a taspine level of 5420 ppm with a dry product yield of 34 kg.

The resin recovered from a second or repeated chromatography step was performed in the same manner described immediately above to recover 279 kg of resin wash. The 30% acetone resin was dried to yield 18.0 kg of dry product.

5.1.7 DRYING PHASE

The resulting enriched extract was tray dried at a depth of one-quarter inch at 60°C for 138 hours. The resulting material was tested and had a moisture content of 6.24 %. The material was collected, double bagged in polyethylene. A desiccant was inserted between the first and second bags. The resultant yield was an enriched proanthocyanidin extract 90 kilograms of a red amorphous powder, the powder having a taspine content of 0.02%) and a proanthocyanidin content of 70.3%.

5.1.8 CHARACTERIZING THE CONCENTRATE

The phenolic content, proanthocyanidin weight percent, the taspine levels and the moisture content of the dried extract were determined by the following assays:

5.1.8.1. Total Phenolic Content

The percentage of total phenolic material in the extract from Croton latex was determined using the method of Folin-Ciocalteau. Solutions were prepared by dissolving dried samples of the concentrated proanthocyanidins in deionized water (400 mg/100 ml). A 1:1000 dilution of the above solution was made by pipetting 0.1 ml into a Class A 100 ml volumetric flask. Appropriate amounts of gallic acid stock was transferred into 100 ml Class A volumetric flasks and diluted with deionized water to the appropriate mark to create 0.005 mg/ml; 0.0025 mg/ml; and 0.00125 mg/ml standards. After a reaction time of 30 seconds and no longer than 8 minutes in 5 ml of 2.0 N Folin-Ciocalteau phenol reagent (Sigma catalog number F9252 or equivalent), 15 ml of 20% sodium carbonate solution was added to each sample and standard flask. Each flask was diluted to volume (water was added to the mark of each flask). Absorbance at peak maximums (750-760 nm) was recorded after the solutions are allowed to sit for 2 hours. Results showed that extracts generally contained 50 to 70 mg of gallaic acid equivalents per 100 mg of extract. The concentrate prepares in Sections 5.1.1 through 5.1.7 was about 55% w/w GAE.

5.1.8.2 Proanthocyanidin Polymer Weight Percentage

Prepare samples & standards

The weight percentage of a proanthocyanidin polymer ("PP") as described in USP 5,211,944 and USP 5,494,661, incorporated by reference herein, is determined in the enriched proanthocyanidin extract of the present invention. 2.5 mg/ml 20% methanol/80% water solutions ("20% methanol") were prepared by weighing out 500 mg of PP and sample, which was dissolved in a 50 ml volumetric flask ("first PP solution" and "first sample solution", respectively). These first PP and sample solutions were diluted (1:3) with 20% methanol.

A solid phase extraction cartridge containing 100 mg of PEI-300 angstroms-50 µm silica anion exchange chromatography medium (purchased from Millipore) was used to purify PP from the sample. The resin was equilibrated prior to loading with the sample by a first wash of 1 ml of 100% methanol and then 1 ml of water. 1 ml of sample or standard was then loaded onto the cartridge, followed by a wash with 0.75 ml of 50% acetonitrile; a wash of 0.75 ml of 100% acetonitrile. The proanthocyanidin polymers were eluted off the column with 1 ml of N,N, dimethylformamide solution (1 ml of DMF containing 1 M $LiNO_3$ and 0.01 N HCl). The effluent was collected and 10 µL were injected into an High Performance Liquid Chromatography system ("HPLC"). A reverse-phase HPLC column (Waters, Symmetry Shield, Model RP-8, 5mm, 3.9 x 150 mm Part No. WA200675) was used. The following conditions were employed for the separation and quantification:

Column temperature: ambient temperature;

Mobile Phase and Gradient Program:

Solvent A : 0.1% Trifluoroacetic acid ("TFA") (v/v)

Solvent B: methanol

Minutes	Percent	
	Solvent A	Solvent B
	0.1% TFA in water	Methanol
0	80	20
14	80	20
17	30	70
25	30	70
35	80	20

Run time was 35 minutes at a 1.0 ml/minute flow rate. The injection volume was 10 µL. Detection was using ultraviolet light at 280 nm (Band width BW=4), reference wavelength subtracted at 550 nm (BW = 100). An HPLC chromatogram is generated with a prominent peak detected at 280 nm. By comparing the area under the 280 nm peak for a given sample with that of the PP standard, the amount of PP present (70.34%) in the enriched proanthocyanidin concentrate was determined for the concentrate prepared in Section 5.1.1 through 5.1.7.

5.1.8.3 Taspine Content

Three solid PP reference standards that contain taspine at 100 ppm; 500 ppm and 1000 ppm were prepared.

5.1.8.3.1 Solid Sample Analysis

100 ± 2 mg of standards and samples were weighed out and 2 ml of deionized water was added, the pH of the test solutions was adjusted to 9.7-10.7. 4 ml of dichloromethane was added. The layers were vortexed and then allowed to separate. A Pasteur pipette tube was prepared with 4-6 cm of anhydrous sodium sulfate. The organic layer was carefully removed and passed through the anhydrous sodium sulfate to remove any water from the organic solution. Spectrums of the standards and the sample extracts were obtained from 220 to 420 nm and an absorbance peak at 348 nm ± 5 nm was ascertained. If the absorbance of the sample was larger than the absorbance obtained for the 1000 ppm standard, a 1 to 10 dilution of the sample was made in DCM, by mixing 1 ml of the sample with 9 ml of DCM. The absorbance of the diluted sample was determined. The slope of the line when plotting absorbance of the standards at 100, 500 and 1000 ppm (y-axis) vs. concentration in ppm of the standards (x-axis) was determined. The concentration of the taspine in the samples was calculated using the following formula:

$$[\text{taspine}]\ (\text{ppm}) = \frac{\text{absorbance of sample} \times \text{dilution factor}}{\text{slope of standards line}}$$

5.1.8.3.2 Liquid Sample Analysis

100 ± 2 mg of standards was weighed and 2 ml of deionized water was added. 500 µl of the liquid samples were pipetted into 10 ml sample vials. 2 ml of deionized water was added to all standards, an empty vial for solvent blank and 1.5 ml to all liquid samples. The pH of the test solutions was adjusted to 9.7 - 10.7. 4 ml of dichloromethane was added, mixed and the layers were allowed to separate. Pasteur pipette

tubes with 4-6 cm of anhydrous sodium sulfate were prepared. The organic layer and was carefully removed and passed through the anhydrous sodium sulfate to remove any water from the organic solution. The spectrums of the standards and the sample concentrates from 220 to 420 nm and the absorbance peak at 348 nm ± 5 nm was obtained. If the absorbance of the sample was larger than the absorbance obtained for the 1000 ppm standard, a 1 to 10 dilution of the sample was made in DCM, by mixing 1 ml of the sample with 9 ml of DCM. The absorbance of the diluted sample was determined. The slope of the line when plotting absorbance of the standards at 100, 500 and 1000 ppm (y-axis) vs. concentration in ppm of the standards (x-axis) was determined. The concentration of the taspine was then calculated using the following formula:

$$\text{taspine (ppm)} = \frac{\text{absorbance of sample x dilution factor x 100}}{\text{slope of standards line x dry weight of liquid sample (mg)}}$$

The taspine content for the proanthocyanidin concentrate prepared according to Sections 5.1.1 through 5.1.7 was 0.2%

5.1.8.4. Moisture Content

The moisture content of the sample was determined according to the methodology of using a Karl Fischer Titrator (Metroohm 701 KF Titrino Karl Fischer Titrator sold by Brinkman Instruments, Inc. of Westbury, NY, set with the following parameters:

Extraction Time:	-10 seconds
Stop criterion	Drift
Stop Drift	100µL
Stop V	30.0 ml
Start V	0.0 mL
Max rate	10 ml/min.
Min. volume increment	2 µL

Calibration of the Karl Fischer reagent (Aquastar™ Composite 5K reagent sold by EM Science (catalog No. AX 1698FN-1) is performed by titrating an accurately weighed amount (25 µL of purified water)

Analysis of the sample

Over 500 mg of the sample were quickly ground to a fine powder into a fine powder using a mortar and pestle. 150 mg of the powdered sample was tested on the titrator. The % moisture was calculated by the instrument as 6.24%.

5.2 ENTERIC COATED TABLETS

A method for formulating the proanthocyanidin polymer composition with a diluent as enteric coated tablets is described below. For each 600 mg tablet, an amount of the proanthocyanidin concentrate prepared according to the methods of Section 5.1 having the equivalent to 250 mg of pure (97%) or substantially pure (95%) proanthocyanidin compound described in USP 5,211,944 was used. For example, 350 mg or more of the proanthocyanidin polymer concentrate prepared according to this invention was mixed with 3 mg colloidal silicon dioxide and mixed in two parts for about 2 minutes. Glyceryl monostearate, sodium starch glycolate and then a sufficient mass of microcrystalline cellulose ("AVICEL™ M 200") was mixed with the other ingredients to bring the total mass to 600 mg. The resulting mix was compressed on a rotary tablet press using 0.6115 X 0.305 inch oval standard concave punches. An enteric coating mixture prepared for 100 kg of tablets from 246.9 grams EUDRAGIT™ L30 D-55, 7.5 grams triethylcitrate, 18.6 grams talc and 227.1 grams distilled water. The tablets are then placed in a perforated pan coater or a fluid bed coater (*e.g.*, the "AROMATIC STREA 1™" system). When using the "AROMATIC STREA 1™" system, the tablet bed is warmed for 3 minutes under the following conditions: nozzle size 0.8 mm; diffuser plate 8 % opening; drying temperature setting 40¡ C; fan capacity at 10 and atomizing air at 1 bar. The enteric coating formulation is sprayed using a peristaltic pump (sold by Watson-Marlow) under the following conditions: nozzle size 0.8 mm; diffuser plate 8 % opening; suspension delivery rate 5, drying temperature setting 40¡ C; fan capacity at 10, atomizing air at 1 bar, drying temperature 34° C and fan capacity at 10. After the desired amount of coating suspension has been sprayed, the peristaltic pump is turned off, the atomizing air is turned off, and the drying temperature is adjusted to 45-50° C. The fan air is adjusted so that the table bed has minimum motion and blowing is continued for 30 minutes. The drying temperature is adjusted to 23° C until the outlet temperature has reached 32° C or below.

A batch formula prepared according to the methodology was prepared as follows:

Table 1

Batch Formula

weight	weight %	ingredient
400 mg	66.7%	milled proanthocyanidin concentrate
3 mg	0.5%	colloidal silicon dioxide
21 mg	3.5%	glyceryl monostereate
48 mg	8.0%	sodium starch glycolate
128 mg	21.3%	microcrystal cellulose
600 mg	100%	total

Fresh coating dispersion according to the following formula was prepared for each 100 kg of table cores. It provides more than the actual quantity needed.

Table 2

weight	weight %	ingredient
24.69 kg	49.4%	methacrylic acid copolymer dispersion (Eudragit® L 30D-55)
22.71 kg	45.4 %	purified water
0.75 kg	1.5%	triethyl citrate
1.86 kg	3.7%	Talc
50.01 kg	100%	

After coating, since water evaporates during the coating, the dried weight per tablet after coating was as set forth in Table 3 below.

TABLE 3 enteric coated tablet

600 mg	92.6%	core tablet
35.5 mg	5.5%	methacrylic acid copolymer (Eudragit® L 30D-55)
0 mg	0%	Purified water
3.6	0.6%	Triethyl acetate
8.9	1.4%	Talc
648.0	100%	total

The invention described and claimed herein is not to be limited in scope by the specific embodiments herein disclosed since these embodiments are intended as illustrations of several aspects of the invention. Any equivalent embodiments are intended to be within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described therein will become apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

All publications cited herein are incorporated by reference in their entirety.

What is claimed is:

1. A method for preparing an enriched proanthocyanidin concentrate from latex from *Croton* ssp latex, comprising:

(a) precipitating *Croton ssp* latex by adjusting the pH of said latex;

(b) removing precipitated residue from said precipitated latex to produce a filtrate;

(c) concentrating said filtrate to obtain a retentate; and

(d) drying said retentate, said retentate essentially free of anti-foaming agents.

2. The method of claim 1, wherein said anti-foaming agents are C_1-C_8 alcohols.

3. The method of claim 1 further including the step of removing additional taspine from said retentate by contacting said retentate with a chromatographic media which removes taspine from said retentate.

4. The method of claim 3 wherein said chromatographic material is an ion exchange resin.

5. A dietary supplement for administration to mammals suffering from gastric disorders comprising a proanthocyanidin enriched concentrate obtained from *Croton ssp* latex by a process which comprises:

(a) precipitating *Croton ssp* latex by adjusting the pH of said latex;

(b) removing precipitated residue from said precipitated latex to produce a filtrate;

(c) concentrating said filtrate to obtain a retentate; and

(d) drying said retentate, said retentate essentially free of anti-foaming agents.

6. The dietary supplement of claim 5, wherein said anti-foaming agents are C_1-C_8 alcohols.

7. The dietary supplement of claim 5 wherein said concentrate is obtained by a process further including the step of removing additional taspine from said retentate by

contacting said retentate with a chromatographic media which removes taspine from said retentate.

8. The dietary supplement of claim 7 wherein said chromatographic material is an ion exchange resin.

9. The dietary supplement of claim 5 wherein said proanthocyanidin enriched extract is formulated for delayed delivery to the colon.

10. The dietary supplement of claim 5 wherein said proanthocyanidin enriched extract is enterically formulated to protect said proanthocyanidin enriched extract from the effects of stomach acid and pepsin.

11. A dietary supplement for administration to mammals suffering from gastric disorders comprising a proanthocyanidin enriched concentrate, said concentrate comprising at least 35 % proanthocyanidin polymers, and less than 1 % taspine, wherein said concentrate is obtained from *Croton ssp* latex by a method which comprises:

(a) precipitating Croton plant latex by adjusting the pH of said latex to between 6.5 to 7.5;

(b) filtering said precipitated latex to obtain a filtrate;

(c) ultrafiltering said filtrate through a 500 d to 3 kd membrane to obtain a retentate;

(d) removing taspine from said retentate by ion exchange chromatography to obtain a retentate filtrate; and

(e) tray drying said retentate filtrate to obtain a dried retentate filtrate, said retentate filtrate essentially free of anti-foaming agents.

12. The dietary supplement of claim 11, wherein said step of removing taspine includes the steps of:

contacting said retentate with an ion exchange media; and

removing the ion exchange media to obtain a retentate filtrate.

13. The dietary supplement of claim 11, further including the step of adding an antimicrobial agent before the tray drying step.

14. A process for preparing an enriched proanthocyanidin material from Croton plant latex, comprising:

(a) precipitating Croton plant latex by adjusting the pH of said latex to between 6.5 to 7.5;

(b) filtering said precipitated latex to obtain a filtrate;

(c) concentrating by ultrafiltration through a 500 d to 3 kd membrane to obtain a retentate;

(d) removing taspine from said retentate by ion exchange chromatography to obtain a retentate filtrate;

(e) tray drying said retentate filtrate to obtain a dried retentate filtrate, said retentate filtrate essentially free of anti-foaming agents.

15. The process of claim 14, further including the step of adding an antimicrobial agent before the tray drying step.

16. A dietary supplement comprising a core table, said core table comprising:

(a) 40-99.5 % proanthocyanidin concentrate, said concentrate obtained from *Croton ssp* latex by a method which comprises (i) precipitating Croton plant latex by adjusting the pH of said latex to between 6.5 to 7.5; (ii) filtering said precipitated latex to obtain a filtrate; (iii) concentrating said filtrate by ultrafiltering through a 500 d to 3 kd membrane to obtain a retentate; (iv) removing taspine from said retentate by ion exchange chromatography to obtain a retentate filtrate; and (v) tray drying said retentate filtrate to obtain a dried retentate filtrate, said retentate filtrate essentially free of anti-foaming agents;

(b) 0 -5% glidant;

(c) 0.5-10% lubricant;

(d) 0-10% disintegrant; and

(e) 0.5%-59.5% filler.

17. The dietary supplement of claim 16, wherein said core tablet further comprises an enteric coating, said enteric coating comprising (a) 50-80% enteric copolymer; (b) 0.05 to 5 % plasticizer; and (c) 15 to 49.95% filler.

18. A dietary supplement comprising a core table, said core table comprising

(a) 60-70 % proanthocyanidin concentrate, said concentrate obtained from *Croton ssp* latex by a method which comprises (i) precipitating Croton plant latex by adjusting the pH of said latex to between 6.5 to 7.5; (ii) filtering said precipitated latex to

obtain a filtrate; (iii) concentrating said filtrate by ultrafiltering through a 500 d to 3 kd membrane to obtain a retentate; (iv) removing taspine from said retentate by solid phase extraction to obtain a retentate filtrate; and (v) tray drying said retentate filtrate to obtain a dried retentate filtrate, said retentate filtrate essentially free of anti-foaming agents;

(b) 0.25 -1% colloidal silicon dioxide;

(c) 2-5% glyceryl monostereate;

(d) 5-10% sodium starch glycolate; and

(e) 14% to 32% microcrystalline cellulose.

19. The dietary supplement of claim 18, further including an enteric coating, said enteric coating comprising

(a) 60-78% copolymer dispersion;

(b) 0.05 to 1 % triethyl citrate; and

(c) 12.5 to 39.95% talc.

20. A dietary supplement for administration to a mammal suffering gastrointestinal distress comprising:

a proanthocyanidin polymer enriched extract; and

at least one additional herbal agent selected from the group consisting of ginger and cinnamon.

21. The dietary supplement of claim 20 wherein said proanthocyanidin polymer enriched extract is formulated for delayed delivery to the colon.

22. The dietary supplement of claim 21 wherein said proanthocyanidin enriched extract is enterically formulated to protect said proanthocyanidin enriched extract from the effects of stomach acid and pepsin.

23. The dietary supplement of claim 22, in which said enterically formulated proanthocyanidin enriched extract further includes an enteric coating, said enteric coating is comprised of a methacrylic acid-methacrylic acid ester copolymer with acid ionizable groups.

24. The dietary supplement of claim 20 further comprising peppermint oil.

25. The dietary supplement of claim 24 wherein said proanthocyanidin enriched extract and said peppermint oil are formulated for delayed delivery to the colon.

26. The dietary supplement of claim 25 wherein said proanthocyanidin enriched extract and said peppermint oil are enterically formulated to protect said proanthocyanidin enriched extract and said peppermint oil from the effects of stomach acid and pepsin.

27. The dietary supplement of claim 26, in which said enterically formulated proanthocyanidin enriched extract and said peppermint oil further include an enteric coating, said enteric coating is comprised of a methacrylic acid-methacrylic acid ester copolymer with acid ionizable groups.

28. The dietary supplement of claim 20, wherein said at least one additional herbal agent is formulated for delivery to the stomach.

29. The dietary supplement of claim 20, wherein said at least one additional herbal agent comprises ginger and cinnamon.

30. The dietary supplement of claim 20 wherein a weight ratio of proanthocyanidin enriched extract to said at least one additional herbal agent is between 0.333 and 1.0 to 1.0.

31. The dietary supplement of claim 20, wherein a weight ratio of proanthocyanidin enriched extract to said peppermint oil is between 0.5 and 1.5 to 1.